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OF THE

Emerging Challenges and Opportunities in Horticulture Supporting Sustainable Development Goals

ISH 2018

(Kuta, Bali, Indonesia 27-30 November 2018)

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FOREWORD

The International Symposia on Horticulture (ISH) was held for 4 days from 27 until 30 November 2018. This Symposia was organized by Indonesian Center for Horticulture Research and Development (ICHORD) under Indonesian Agency for Agricultural Research and Development (IAARD) Ministry of Agriculture, and was supported by Australian Center for International Agricultural Research (ACIAR), Indonesian Horticulture Association (PERHORTI) and Indonesian Agronomy Association (PERAGI). Theme of ISH was "Emerging Challenges and Opportunities in Horticulture Supporting Sustainable Development Goals". The outputs of the Symposia were (1) elevation a number of new ideas of horticulture innovation development, (2) spread information related to technology of horticulture innovation among horticulture scientists and practicians, and (3) increase collaboration among the various parties to develop agri-horticulture networks.

This event was attended by approximately 200 participants from various institutions such as from Universities, Government Officers Private Company, and also horticulture observers. They came from some countries, Indonesia, Japan, Philippines, Thailand, Malaysia, Brazil, Iran, India, South Korea, New Zealand, Ukraine and Germany. Around 132 participants were oral presenter and 71 participants were poster presenter. The symposia were enriched by 23 resource persons who shared their knowledge which were 10 from Indonesia; and 13 resources persons were from International Institution such as FAO, ACIAR, Agriculture Technology and Knowledge Extension Iran, Adelaide University, Queensland University, Humbolt University, Gent University, Sizuoka University, Leibniz University Hannover, CIRAD, and Sakata Seed Company.

This proceeding contains 5 main topics such as tropical and sub-tropical fruits, vegetables, ornamental plants, socio economics studies and integrated crop managements, which have been selected and reviewed by editors.

In this opportunity, I would like to deliver a highly appreciation to the resource persons for willingness to share their knowledge, to all participants and organizing committee for all their contributions. Hopefully this proceeding can bring benefits to all of us and for the development of horticulture innovations.

Thank you

Dr. Hardiyanto, MSc
Director of ICHORD and President of ISH 2018

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PROMOTE THE EXOTICS OF TROPICAL AND SUB TROPICAL FRUITS TO ENHANCE COMPETITIVENESS AND PENETRATION INTO MODERN MARKETS

Controlling Diplodia Using Trichocompost and Sulfur-Calcium to Improve Siam Citrus Fruit Yield in Bali

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Abstract

Diplodia is one of the main diseases which frequently infects siam citrus plants in Bali. The aim of this research was to obtain the optimum composition of trichocompost and sulphur-calcium suspension as a fungicide treatment to suppress the infection of Diplodia disease and increase the fruit yield. The research was conducted in Kintamani, Bali from January-December 2016 in which different compositions of trichocompost and sulfur-calcium were used. The results revealed that the treatment of 60 kg trichocompost coupled with 125 g sulphur-calcium suspension was able to reduce the percentage of Diplodia infection and suppress disease intensity up to 21.21% and 2.05%, respectively. It was also demonstrated the highest contribution to the emergence of new shoots (40.96) and the appearance of fruits set (89.30) which was not significantly different from treatment of 40 kg trichocompost and 125 g sulphur-calcium suspension i.e. 35.8 and 77.2, respectively. The treatment of 60 kg trichocompost and 125 g sulphur-calcium suspension increased the yield by 6.1 pieces significantly higher than the farmers' conventional treatment.

Therefore, application 40-60 kg trichocompost with addition sulphur-calcium suspension per plant significantly reduces the percentage of Diplodia as well as increasing yield components.

Keywords: Diplodia, trichocompost, citrus

Introduction

Horticultural products are commodities which possess high economic value and market demand. The contribution of horticultural commodities to the development of the agricultural sector shows an increasing trend, which is indicated by the escalation of macro indicators such as export volume, employment in the supply chain points, and farmer exchange rates, annually [1].

Further, Citrus is one of the most traded fruits globally due to its relatively affordable price, long-term shelf life and high nutrient content. Siamese citrus is one of the leading fruit commodities in Bali, Indonesia and the dominant production center is in Kintamani, Bangli District [2].

Scaling up the production and improvement of the quality are required in order to avoid economic losses in citrus plantation farming. Based on the data from the Central Bureau of Statistics of Indonesia (BPS), within the last five years, the production of Bali Siamese citrus was fluctuated between 63,426 to 119,030 metric tons per year [2]. Various efforts have been implemented to increase the production and quality. However, pest and disease attacks remain the major obstacles in realizing this goal. One of the most harmful is Diplodia stem-end rot as the main disease that attacks citrus which is associated with fungi *BotryoDiplodia theobromae Pat*. It is polyphagic and attacks various plants, therefore the source of infection will always exist in the environment prevalently [3].

Diplodia disease in citrus plants can be observed by looking at the symptoms found in the stem.

The symptoms are characterized by the discharge of yellow or foamy liquid and cracking of the skin due to pathogens activities which cause the stem to rot. Diplodia disease or often referred to as Diplodia gummosis because it reacts releasing gum [4]. Penetration causes the plant to react by releasing yellow defense substances such as gummosis which is released by plants as a form of reaction after an attack of pathogens in the tissue, and it is produced to localize and to restrict the growth of the pathogens [5]. Gummosis that comes out from the skin surface of plant tissue shows an advanced level of attack. In addition to attacking citrus plants this fungus also attacks mango [6], cashew nuts [7], pomes, stone fruits [8] and other horticultural plants [9]. Once the fungi attack a citrus plant, it may result in the death of twigs, branches, plant stems, and even cause plant death. The source of pathogenic inoculum is able to survive in seeds, fruit peels, plant stems, and healthy tree branches, yet not found on the ground. Pathogens spread through insect vectors, attaching/wounding, spraying through the air, and splashing water [10].

Biological control can be implemented to tackle the Diplodia infection on horticultural commodities. One biological agent which has been proven to be effective against the infection of *BotryoDiplodia Theobroma* is *Trichoderma* species. A study, employing several strains of *Trichoderma,* demonstrated efficacy against soil borne pathogenic fungi [11]. In terms of application on plants, compost modification by addition of the biological agent i.e., *Trichoderma* spp enhances the efficacy of the disease control as well as increasing the value addition [12].

The farmers' practice generally rely on the use of chemical pesticides, since it is faster to obtain results and more practical. However excessive or uncontrolled doses of application risk the environment. Based on these conditions, studies need to be conducted implementing local resource-based technology applying compost with the addition of *Trichoderma* to make a substance called *trichocompost* which is combined with sulphur – calcium suspension as root drench. The purpose of this study was to investigate the effects of calcium – sulphur suspension and *trichocompost* against Diplodia diseases in Siamese citrus in Kintamani.

Methodology

Location and time

The study was conducted in the field at Bonyoh Village, Kintamani Sub-District, Bangli District from January-December 2016. The impact after treatment was observed in the second year (2017).

Materials

In the making of trichocompost, cow manure and vegetable waste were mixed and homogenized followed by making layers of dung about 20 cm thick. Dolomite lime was added on top of each layer to maintain the normal pH of the soil. In each layer, a BEKA decomposer (Indo Acidatama, Indonesia) and molasses solution was sprayed, the mixture was then stirred to evenly distribute. Fermentation was conducted for 7 days and the Trichoderma spp inoculum added in the day 7, then the fermentation process was continued until day 21. Trichoderma spp isolates were obtained from The Pest and Diseases Laboratory of Udayana University, there were isolated according to Dwiastuti, Fajri, & Yunimar [13] with modification. In brief, the culture was incubated from lyophilized culture stock for 6-7 days at 20oC with the number of spores around ~1x107 CFU/ml before application.

Materials used to make sulphur-calcium suspension were sulphur powder (Indosulfur Mitra Kimia, Indonesia), calcium hydroxide in the form of slaked lime (Petrokimia, Indonesia), and clean water with the concentration in the mixture of 1:2:10, respectively. To make the suspension, sulfur powder was boiled and then it was added homogenously with the slaked lime. The final product of suspension was reddish yellow in colour. Application treatments of sulphur-calcium suspension in the field were conducted by initially cleaning the stems, branches, and twigs of the trees. With the stems where the gum occurs, the bark was peeled off to facilitate application of root drench suspension. Calcium – sulphur suspension as root drench was sprinkled on the stems and branches of the plant at the beginning and end of rainy season. While the treatment of trichocompost fertilization was given to citrus plants that were 8-10 years of age and which showed symptoms of diplopia attacks.

Experimental Design

The design used was Random Block Design with two different factors, i.e., fertilization (P) and calcium – sulphur suspension treatment (D) with the details as follow:

- P0: fertilization with conventional way
- P1: fertilization with *trichocompost* 20 kg/tree/year
- P2: fertilization with *trichocompost* 40 kg/tree/year
- P3: fertilization with *trichocompost* 60 kg/tree/year
- D0: without calcium – sulphur suspension
- D1: with calcium – sulphur suspension 125 gram/tree

Each treatment used 5 sample trees in 5 sub-groups of farmers. Parameters were observed i.e., existing conditions, the percentage of affected plants, the intensity of the disease, the number of new emergence shoots (stem), and the number of fruits set.

The infected plant percentage was calculated with the following formula:

$$\% PI = \frac{n}{N} \times 100\% \qquad (1)$$

Where PI = Percentage of infection; n = number of infected plants; N = total observed plants
In addition, the disease intensity was determined with:

$$DI = \frac{\sum_{i=0}^{i}(ni.vi)}{N.V} \times 100\% \qquad (2)$$

Where DI = diseases intensity; ∑ = the sum of the number attacked leaves multiply by the score of each category; ni = number of leaves attacked in each category; vi = score in each category; N = highest score in each category; V = number of total leaves observed.

To measure the diseases' intensity the following score or scale of each category was used:

- 0 = no attack at all
- 1 = very mild attack (0-10% of leaf surface was attacked)
- 2 = mild attack (10-30% of leaf surface was attacked)
- 3 = moderate attack (30-50% of leaf surface was attacked)
- 4 = severe attack (50-75% of leaf surface was attacked)
- 5 = very heavy attack (75-100% of leaf surface was attacked)

Data Analysis

Data was analysed by analysis of variance (ANOVA). If the test treatment showed a significant difference it was followed by the Duncan test.

Results and Discussion

Existing Conditions

The location of the study was in Subak Kelaan Mertasari, Bonyoh Village, Kintamani Sub-District, Bangli District. Based on observations, there were 23 farmers as *subak* (traditional farmers organization) members and all of them cultivated Siamese Citrus. The average land ownership of each farmer is 0.56 ha. The number of productive citrus plants on average was 412.5 trees and plants awaiting to yield were about of 118 trees.

Currently, land use in Bonyoh Village consists of moor (fallow land) and mixed gardens, upland is a dryland for staple food farming that relies solely on rainwater with various plants cultivated e.g., corn, sweet potato, cassava, chili, chickpeas, and squash, covering an area of 33.07 ha (9.41%). The soil type is dominated by Andisol soil with a dusty clay texture. Fertilizers commonly used in the cultivation of siam citrus are manure compost (organic fertilizer) and chemical fertilizers. The manure was unprocessed chicken and cow manure with irregular doses or application. Meanwhile, to control pest and disease attacks on siam citrus the farmers used chemical pesticides.

The Results of the Study

The initial condition of plants (when starting treatment) all showed the percentage of plants attacked as 100%. The average percentage of early infected plants showed conditions that were not significantly different, 100% of Siamese citrus plants attacked by Diplodia disease (data not shown). While the intensity of the initial disease intensity which also showed no significant difference between the treatments tested, ranging from 0.56-0,72% which is showed in Table 1.

Table 1. Infected plants percentage and initial diseases intensity (%) of Diplodia disease

Treatments	Initial diseases intensity (%)
P0D0 (Conventional fertilization without calcium -sulphur suspension)	0.70±0.12a
P0D1(Conventional fertilization with calcium -sulphur suspension)	0.62±0.18a
P1D0 (*trichocompost* 20 kg/tree/year without calcium -sulphur suspension)	0.72±0.04a
P1D1 (*trichocompost* 20 kg/tree/year with calcium -sulphur suspension)	0.54±0.09a
P2D0 (*trichocompost* 40 kg/tree/year without calcium -sulphur suspension)	0.62±0.15a
P2D1 (*trichocompost* 40 kg/tree/year with calcium -sulphur suspension)	0.56±0.25a
P3D0 (*trichocompost* 60 kg/tree/year without calcium -sulphur suspension)	0.56±0.17a
P3D1 (*trichocompost* 60 kg/tree/year with calcium -sulphur suspension)	0.62±0.18a

Note: For each sample, means within each column followed by a different letter are significantly different ($p<0.05$).

Based on the results of the analysis, there was a real interaction due to the treatment of *trichocompost* and calcium – sulphur suspension as showed in Table 2. Which shows that the provision of 60 kg/tree/year *trichocompost* fertilizer coupled with calcium – sulphur suspension treatment gave the biggest contribution in suppressing infection of 21.21%. This result significantly gives positive effect compared to other treatments. As for the existing farmers' treatment employing raw manure compost and commercial chemical fertilizers; it showed the highest percentage of infection at about 76%.

Table 2. Effect of *Trichocompost* fertilization interactions with calcium – sulphur suspension on the percentage of infection (%)

	Percentage of infection (%)			
Fertilization	Without calcium – sulphur		Calcium-sulphur	
fertilization conventional way	76.10±8.07	a	74.29±11.95	b
fertilization *trichocompost* 20 kg/tree/year	40.00±21.19	d	42.86±17.50	c
fertilization *trichocompost* 40 kg/tree/year	37.14±21.67	e	22.86±7.82	g
fertilization *trichocompost* 60 kg/tree/year	25.71±6.39	f	21.21±16.94	h
CV (%)	20.985			

Description: For each sample, means within each column followed by a different letter are significantly different ($p < 0.05$).

Furthermore, the single application of calcium-sulphur suspension treatment had a significant effect on decreasing disease intensity. The treatment was capable of reducing Diplodia disease intensity by up to 2% lower than conventional treatment without calcium-sulphur suspension (2.64%) (Table 3.).

Table 3. The effect of single Calcium-sulphur suspension on the intensity of final attacks (%)

Treatment	Diseases intensity (%)
without calcium-sulphur suspension	2.64±0.61a
Calcium-sulphur suspension	2.05±0.66b
LSD 5%	0.03
CV (%)	9.49

Note: For each sample, means within each column followed by a different letter are significantly different ($p < 0.05$).

The impact of treatment was also seen in the yield parameter of the number of emerging shoots.

As can be observed in Table 4 the interaction between the treatment of *trichocompost* and Calcium-sulfuric suspension increases the number of new shoots. Fertilization *trichocompost* 60 kg/tree/year with addition of calcium-sulfuric suspension indicated the highest contribution in supporting the emergence of new shoots 40.96 stems. Compared to the number of new shoots at conventional treatment 13 stems, thus, the application of the correct treatment using *trichocompost* and calcium-sulfuric suspension increases the number of new shoots by 66.79% over conventional treatment.

Table 4. Effect of *Trichocompost* fertilization interactions with Calcium-sulfuric suspension on new shoots (stems)

Fertilization	Number of emerging shoots (pieces)			
	D0 (without calcium-sulphur suspension)		D1 (with calcium-sulphur suspension)	
P0 (fertilization conventional way)	13.00±1.00	h	13.40±1.67	gh
P1 (fertilization *trichocompost* 20 kg/tree/year)	21.40±12.86	f	34.40±19.09	d
P2 (fertilization *trichocompost* 40 kg/tree/year)	34.00±8.37	e	39.15±15.01	ab
P3 (fertilization *trichocompost* 60 kg/tree/year)	35.80±11.45	c	40.96±14.74	a
CV (%)	17.84			

Note: For each sample, means within each column followed by a different letter are significantly different (p<0.05).

Looking at Table 5, the application of *trichocompost* and calcium-sulphur fertilizers was the major influence in increasing the appearance of new citrus fruits set. The interaction of 60 kg *trichocompost* and calcium-sulphur resulted 89.30 fruits set, while 40 kg *trichocompost* and calcium-sulphur resulted in 89.16 fruit sets, both treatments are not significantly different in supporting the emergence of fruit set statistically. Therefore, the first treatment was able to increase the emergence of young fruit by 75.81% compared to the current farmers' practice. While the second treatment contributed to 75.77% higher emergence of fruits set compared to the conventional farmers' treatment of citrus plants with raw chicken manure and chemical fertilizer.

Table 5. Effect of *trichocompost* fertilization interactions with Calcium-sulphur suspension on young fruits set

Fertilization treatments	Fruit set (pieces)			
	D0 (without calcium-sulphur suspension)		D1 (with calcium-sulphur suspension)	
P0 (fertilization conventional way)	21.60±4.51	g	24.60±8.17	f
P1 (fertilization *trichocompost* 20 kg/tree/year)	33.40±12.97	e	54.60±14.33	d
P2 (fertilization *trichocompost* 40 kg/tree/year)	58.20±14.07	c	89.16±6.68	a
P3 (fertilization *trichocompost* 60 kg/tree/year)	77.20±14.15	b	89.30±7.01	a
CV (%)	16.180			

Note: For each sample, means within each column followed by a different letter are significantly different (p<0.05).

The application of the treatment between *trichocompost* and calcium-sulphur suspension statistically increases the yield of citrus fruits as illustrated in Table 6. The treatment of fertilization *trichocompost* 60 kg/tree/year coupled with calcium-sulphur suspension shows the biggest contribution in increasing crop yield up to 6.10 kg per plant significantly higher than other treatments, statistically. Compared to yield of farmers current practice without any addition of *trichocompost* and calcium-sulphur, the P3D1 able to boost the fruit yield per plant more than 5-fold.

Table 6. Effect of *trichocompost* fertilization interactions with calcium-sulphur suspension on crop yields

	Yield (kg)	
Fertilization treatments	D0 (without calcium-sulphur suspension)	D1 (with calcium-sulphur suspension)
P0 (fertilization conventional way)	1.18±0.54d	1.80±0.84cd
P1 (*trichocompost* 20 kg/tree/year)	3.0±1.154b	3.70±1.15b
P2 (*trichocompost* 40 kg/tree/year)	4.00±1.77b	4.28±1.87b
P3 (*trichocompost* 60 kg/tree/year)	4.10±0.89b	6.10±1.52a
CV (%)	16.65	

Note: For each sample, means within each column followed by a different letter are significantly different ($p<0.05$).

Table 7 provides an overview of the effects of the *trichocompost* and calcium-sulphur suspension application on several soil quality parameters. The three main macronutrients nitrogen (N), phosphorus (P) and potassium (K) composition of the soil are improved remarkably by the addition of *trichocompost* fertilizer. Soil organic carbon component as well as the electric conductivity on the location are enhanced after the treatments by *trichocompost* fertilization. On the other hand, the soil acidity experiences slight decrease after the application of 20-40 kg *trichocompost,* yet the neutral pH is showed in both control and 60 kg *trichompost* sites.

Table 7. Soil quality comparison between conventional and *trichocompost* application sites

Soil Parameters	Treatments			
	(P0)[a]	(P1)[b]	(P2)[c]	(P3)[d]
pH	7.00	6.51	6.80	7.00
Electric Conductivity (mmhos/cm)	0.66	1.25	1.77	1.75
C-Organic (%)	3.51	4.77	12.58	12.23
N-Total (%)	0.23	0.34	0.49	0.54
P-Available (ppm)	31.79	530.01	604.79	558.30
K-Available (ppm)	145.78	891.60	1113.00	953.44

Notes:
[a]P0 (fertilization conventional way)
[b]P1 (fertilization trichocompost 20 kg/tree/year)
[c]P2 (fertilization trichocompost 40 kg/tree/year)
[d]P3 (fertilization trichocompost 60 kg/tree/year)

Discussions

Either addition of 40 or 60 kg of *trichocompost* coupled with calcium-sulphur suspension contributed significantly to suppress the percentage of infected plants and disease intensity of Diplodia, boost the emergence of new shoots and fruit set as well as escalate fruits yield. However, the mode of action of these mechanisms needs to be further investigated.

Trichocompost fertilizer is a fertilizer derived from the remains of the compost that had undergone a perfect decomposition by *Trichoderma* spp. Generally, *trichocompost* fertilizer contains three important substances for plants, i.e. nutrients, organic matter, and *Trichoderma* spp.

Furthermore, *Trichocompost* fertilizer contains complex nutrients both macro and micro nutrients.

Compared to another study by Bernal-Vicente, Pascual, Tittarelli, Hernández and Diaz-Vivancos [14], the macronutrients (N,P,K) in the location of the experiment in Kintamani was comparable. Especially in P and K available on the soil, as it can be observed in the Table 7 that the P and K is about 0.5% while in the Bernal-Vicente *et al*., [14] is containing NPK at almost the same value.

The second ingredient is organic matter which is part of the soil that came from the remains of plants and animals found in the soil that continue to undergo changes due to physical, chemical and biological factors [15]. The function of an organic material is to improve soil properties, including physical, chemical and biological properties. Physical, chemical, and biological properties of soil which are in good condition stimulate plant growth and development because they can stimulate root growth, increase nutrient availability for plants, and increase soil microbial population [16].

The third component is the fungal inoculum *Trichoderma spp*. which is a biological antagonistic controlling agent, serves as a preventive or competitive effect on plant disease infection, biofertilization and stimulation of plant defense [17, 18]. In Addition to antagonistic character, *Trichoderma spp* also take a role as a decomposer in the process of organic fertilizer [12]. The application of this fungi to plants is done in anticipation of disease infection. *Trichoderma spp* is a soil-dwelling fungus that can be isolated from plant roots. Besides being a decomposing organism, *Trichoderma spp* work as a plant growth stimulator. *Trichoderma spp* able to accelerates growth and development of plants, especially their ability to produce healthy roots and cause the roots to penetrate deeper in the soil. Deeper roots become more resistant to dry condition [10].

Generally, inferring from the results, regular use of organic fertilizers and biological agents combined with calcium-sulphur suspension provide better soil and plant performance compared to the use of chemicals or synthetic pesticide. Better soil quality supports all aspects of plant growth and development as proven in the results of soil analysis which demonstrates that fertilization *trichocompost* 60 kg/tree/year provides better soil quality in preparing soil nutrient availability, especially N, P and K compounds (Table 7.).

The results of another study demonstrated that the application of several *Trichoderma atroviride* isolates provided significant effects to promote the growth of radiata pine seedlings as root, stem and needle biomass stimulation and reduced the Diplodia diseases incidence by inducible defences of POX and terpenes [19]. Another research employs *Trichoderma harzianum* combined with essential oils and compost gives a counteractive effect against crown rot pathogen on peanut, and the author suggested that the inhibition was due to the bioactive compounds synergistic with antagonistic character of *Trichoderma* spp [20].

While in this experiment, the Diplodia inhibition might be caused by the macro and micro nutrients of *trichocompost* that play a role in improving soil structure, facilitating plant root growth, holding water, increasing the biological activity of beneficial soil microorganisms, increasing pH in acidic soils and controlling pest infectious diseases. In this research, Diplodia was controlled by a combination treatment of calcium-sulphur suspension on the stem and the addition of compost at the root of the plant as root drench. A previous experiment conducted by applying lime sulphur coupled with addition of Myco-sin and sanitation environmental control was able to reduce Diplodia on apple plantation about 73% [21].

Calcium and sulphur are both nutrients for the plants, calcium is required to hold together and maintain the cell wall integrity of the plants and offer growth support for tissues. Calcium deficiency does not frequently happen in normal condition of plants, yet it is possible for it to occur on the acidic balance condition of soils [22, 23]. While sulphur, is one of the building blocks of proteins and easily absorbed by the plants [24]. Lack of sulphur contained in the soil might have been occurring due to environmental pollution [25], especially in dry land areas, However, in this case, Kintamani District is a location with heavy rainfall and minor pollutants. Therefore, the combination of calcium-sulphur suspension providing essential elements needed for plants and soil with *Trichoderma* containing compost able to control Diplodia infestation on siam citrus plants provides optimal support the healthy plants growth.

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Genetic Diversity of Pulasan (*Nephelium ramboutan*-ake Bill). Found from Riau Daratan and Riau Kepulauan based on fruit morphology

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Abstract

Pulasan is a close relative of Rambutan. This plant potentially has high genetic varieties due to crossly pollinated. The objectives of the research were to study the similarity and accession grouping in Riau Daratan (area in main island, Sumatera) and Riau Kepulauan (islets in Riau Province) based on morphology diversity. The studies were conducted in two districs in Riau Daratan (Kampar Regency and Kuantan Singingi Regency) and two regencies within Riau Kepulauan (Bengkalis Regency dan Siak Regency). Nutrition contents of the fruit were analysed in Biotechnology Laboratory, in Islamic University of Riau. The study was carried out from January 2017 to September 2017. Fruit characterizations were done on 65 pulasan accessions, based on IPGRI standardization. Similarity coefficient among accessions was analysed using MVSP 32 method. At the level of diversity fruit morphology similarity coefficient reached 73%.

Kampar has the thickest aril. While those of Kuantan Singingi revealed similarity to Bengkalis and Siak. Fruit peel from Riau Kepulauan was thicker than Riau Daratan. From each of location accessed, two different characters of fruit were observed: easily separated aril from the seed and aril stick to the seed.

Keywords: Nephelium ramboutan-ake, morphology fruit, Riau-kepulauan, Riau-daratan

Introduction

Pulasan (*Nephelium ramboutan*-ake Leenh. Bill.) belongs to group of trees producing fruit and seed. There are 22 species of the genus *Nephelium* that have been reported worldwide, 16 species are found in Kalimantan and nine of them are edible (Sieberg 1991; Kuswandi *et al*., 2014). The plant is called ‘pulasan’ since its fruit is easily opened just by wringing (=memulas) its thick yet brittle peel. The well-known varieties of pulasan in Malaysia are P6 dan P28 (*Rukayyah 1999; Syarif J. et al*., *2005; Nurhuda 2013*). Pulasan has high of ascorbic acid and good source of carbohydrate (Burkill *in* Tee *et al*., 1988). The fruit (aril) can be eaten fresh, or being added to ice cream, jam, syrup, or fruit cocktails (*Aphiwat 2010*; *Lim 2013*). The seed is edible after being roasted (the taste is like nut) or can be used as cacao powder.

In Indonesia, this plant is reported to be found in Sumatra, Java and Kalimantan islands (*Rukmana 2002; Lipi 2007*). However, as it is reported by *Ediwirman et al*., *(2011)* the plant is found in cluster and not evenly distributed over the islands as the case with its close relative, rambutan. Riau Province, Riau Daratan is located in the middle of Sumatra island with some part in the shoreline; with the elevation is up to 91 above sea level and two third of it in the area is lower than 5 m above sea level. Riau Province was divided into four regencies. Riau Kepulauan Province consists of several islets with the elevation up to 5 m above sea level. There are eight regencies located in Riau Kepulauan. Most of the soil in Riau is peat and peat minerals (Badan Pusat Statistik 2015).

Based on information gathered from public, pulasan can be found in Bengkalis and Siak Regencies in peat area 2-5 m above sea level with high salinity soil. Pulasan grows in mineral soil in Riau Daratan. Differences in growing environment might result in different plant performance, hence different location often resulted in different genotype. Stoskopf *et al.*, (2009) stated that plant diversity could be measured with morphological marker through observation of morphological characterization. In this study, morphology characterization of mature fruit was assessed to differentiate genetic variation among pulasan grown in different environment.

Differences and varieties of pulasan obtained from Riau Daratan and Riau Kepulauan based on morphological marker are discussed

Materials and Methods

Time and Research Site

Characterization of pulasan fruit was conducted in Biotechnology Laboratory of Agriculture Faculty of The University of Riau. The research was carried out from January 2018 to March 2018.

Materials and Instrument

Materials used were 65 pulasan accessions obtained from pulasan centre in Riau Provinces (Riau Daratan and Riau Kepulauan: Kampar, Kuantan Singingi, Siak and Bengalis Regencies).

Iodine was used to analyse Vitamin C, while titration to analyse total acid was done using NaOH 0,1 N. Instruments used were calliper, camera, analytical scale, blender, hand refractometer, titration instrument, and measuring cylinder.

Methodology

Mature fruit was collected from 4 regencies in Riau Provinces (Kampar, Kuantan Singingi, Siak dan Bengkalis). Characterization of fruit morphology was performed using descriptor standard for rambutan (*Nephelium lappaceum*) published by International Plant Genetic Resources Institute (IPGRI) (2003). Analyses of total dissolved acid and vitamin C used procedure by Sudarmaji *et al.*, (1984), total dissolved acid was determined using 0.1 N NaOH and vitamin C was obtained by using I2 iodine, and Total dissolved solid (^{0}Brix) was determined using handrefractometer. Qualitative characteristics were evaluated by 20 people (panelis). Some of the parameters were graded using score 1-4; 1 being poor, 2 is mild, 3 is good and 4 is very good.

Data were analysed using a nova, followed by Duncan Multiple Rank Test (DMRT) at 5%, using SAS 9.1 program. Coefficient similarity was analysed using MVSP 32.

Results and Discussions

Quantitative Characters

Observation on 10 quantitative characters was performed on fruit with characteristics of mature physiological distributed in 8 districts in Riau Daratan and Riau Kepulauan. Fruit size was measured by its length; which was ranged from 4.78-5.33 cm. Size of fruit originated from Kampar regency (Gunung sahilan, Kampar Kiri dan Kuok) was between 5.13 and 5.33 cm, not significantly different among them, but relatively bigger than those from Kuantan Singingi, Siak and Bengkalis regencies. Fruit from Kuantan Singingi (at average 4.78 cm) was smallest. The size of fruit from Riau Kepulauan (Siak and Bengkalis) was around 4.88-5.14 cm which relatively smaller than those of from Riau Daratan except that of from Kuantan Singingi. The weight of fruit from Riau Daratan and Riau Kepulauan were considered the same, around 36.18 and 40.94 gram.

The peel of pulasan fruit from Riau kepulauan was thicker than that from Riau Daratan. Fruit peel thickness among population within Riau kepulauan was significantly different. The peel of pulasan from Bengkalis was thicker than from Siak. Similar variation among population was also observed regarding the peel thickness of fruit from Riau Daratan. The fruit peels from Kuok and Gunung Sahilan were thicker than those of from Kampar Kiri and Kuantan Singingi; which were the thinnest (0,43-0,38 mm). The weight of fruit peel was in accordance with the peel thickness.

The thicker the peel, the heavier the weight. Pulasan from Bengkalis and Siak with thicker peel, had the heavier weight compared to those of fruit peel from Riau Daratan.

Outer part of the pulasan fruit peel is covered with blunt spines. Spine length of each genotype was varying. Spine length of fruit from Riau Daratan is longer than that of Riau Kepulauan.

However, among populations from Riau Daratan, the spine length of populations from Kampar (3 populations) is longer than that of from Kuansing. Meanwhile, there is no difference of spine length among those of populations within Riau Kepulauan. Length of spines did not describe the density of spines per fruit (Tabel 1).

Table 1. Quantitative characteristics of pulasan fruit morphology based on location distribution in Riau Daratan and Riau Kepulauan

Characteristics (cm, mm, g)	Riau Daratan				Riau Kepulauan	
	Kampar Regency			Kuansing Regency	Siak Regency	Bengkalis Regency
	Gunung Sahilan	Kampar Kiri	Kuok	Sentajo Raya	Siak	Beng Kalis
Fruit size (cm)	5.15 ab	5.31 a	5.33 a	4.78 c	4.88 bc	5.14 ab
Fruit weight (g)	40.94 a	40.71 a	40.52 a	36.18 a	40.61 a	39.99 a
Peelthickness(mm)	0.53 bc	0.38 d	0.59 b	0.43 cd	0.60 b	0.85 a
Peel weight (g)	27.38 abc	25.79 bc	24.76 c	20.83 d	28.37 ab	28.96 a
Spine length (c)	0.65 a	0.57 a	0.66 a	0.51 b	0.42 bc	0.36 c
Number of Spine(2cm^2)	27.80 c	35.23 ab	35.22ab	39.00 ab	34.99 b	39.70 a
Aril weight (g)	12.58 a	11.87 a	12.09 a	11.84 a	9.87 b	9.50 b
Arilthickness (mm)	0.47 b	0.49 ab	0.64 a	0.47 b	0.53 ab	0.59 a
TDS (°Brix)	21.09 a	20.68 a	19.95 a	20.02 a	17.29 b	18.33 b
TDA (%)	5.01 b	6.25 a	4.33 bc	3.60 d	4.14 cd	1.82 e

Means in a column with the same letter are not significantly different by Duncan at 5 % level

The edible part of pulasan fruit is the aril. The mean weight of aril from fruit originated from Riau Daratan was higher than that of from Riau Kepulauan. There was no difference of Aril weight among populations within Riau Daratan. The weight means ranged between 11.84 to 12.58 gram which were significantly different from those of from Siak and Bengkalis (Riau Kepulauan) which were 9.87 and 9.50 gram, respectively.

There were differences in composition of chemical content in each genotype within or among population. The average of vitamin C content from fresh fruit from Kuantan Singingi (Riau Daratan) was 24.44 mg/100 g; which was the highest. Water content of fruit from different location was relatively the same, ranging between 81.63-86.88%.

Table 2. presents the comparison between characteristics of fruit from Riau Daratan to those of Riau Kepulauan. Ratio Total Dissolved Solid (TDS) and Total Dissolved Acid (TDA) indicated the sweetness of the aril. Based on the ratio of TDS over TDA, pulasan fruit from Riau Kepulauan is sweeter than that of from Riau Daratan.

Table 2. Differences of morphological characteristics between pulasan fruit from Riau Daratan and Riau Kepulauan

Characteristics	Zona Riau Province	
	Riau Daratan	Riau Kepulauan
Fruit size (cm)	5.14	5.01
Fruit weight (g)	30.54	40.3
Peel thickness (mm)	0.48	0.72
Peel weight (g)	24.69	28.66
Spine length (mm)	0.59	0.39
Spine number (4 cm^2)	34.31	37.34
Aril weight (g)	12.09	9.68
Aril thickness (mm)	0.58	0.56
Total Dissolved solid = TDS (ºBrix)	20.43	17.81
Total dissolved acid=TDA	4.79	2.92
TDS/TDA ratio	4.26	6.09

Qualitative Characteristics

There were 18 qualitative characteristics which were observed from 8 districts in Riau Daratan and Riau Kepulauan. Twenty people were involved in the assessment; the results were summarized in Table 3 and Table 4. The shapes of pulasan fruit are globose, ovoid, or oblong, with thick peel covered with blunt spines. Spine could affect overall fruit size and attractiveness.

Shape of fruit from Riau daratan mostly globose and ovoid, while those of Riau Kepulauan showed differences between from Siak and Bengkalis. Fruit from Siak mainly globose while fruit from Bengkalis mainly oblong.

Table 3. Qualitative characteristics of pulasan fruit from four districts (growing centre area) in Riau Daratan

Characteristics	Riau Daratan Population (districts)			
	Kampar Kiri	Kuok	Gunung Sahilan	Sentajo Raya
Fruit shape	ob-glob-ov	Ov-Glob	Ov-Glob	Ov-ob-Glob
Fruit rind thickness	Medium	Medium	medium	Thin
Fruit rind colour	Dark red	Dark red	Dark red	Dark red
Uniformity of colour on fruit surface	Uniform	Not-Unif.	Uniform	Not-Unif.
Fruit attractiveness	Good	Good	Good	Intermediate
Fruit quality	Good	Good	Good	Moderate
Spine texture	Stiff	Stiff	Stiff	Stiff
Spine density	Dense	Dense	Dense	Very Dense
Spine colour	Dark red	Dark red	Dark red	Dark red
Spine strength	Strong	Strong	Strong	Strong
Aril colour	c-white	c-White	Dull white	Dull white
Aril thickness	Medium	Thick	medium	medium
Aril texture	Soft	Firm	Firm	Soft
Aril taste	Sweet	Sweet	Sweet	Sweet
Aril flavour	Intermediate	Int- Strong	Intermediate	Intermediate
Aril juiciness	Juicy	Juicy	Juicy	Very juicy
Attachment to frail tossed	Good	Good	Good	medium
Adherence of test a to aril	Tight	Tight	Tight	Interm-Tight

Table 4. Qualitative characteristics of pulasan fruit from four districts (growing centre area) in Riau Kepulauan

Characteristics	Riau Kepulauan population (districts)			
	Sabak Auh	Sei.Apit	Siak Kecil	Bengkalis
Fruit shape	Globose	Globose	oblong	Oblong
Fruit rind thickness	Thick	medium	medium	medium
Fruit rind colour	Dark red	Dark red	Dark red	red
Uniformity of colour on fruit surface	Not Uniform	Uniform	Not uniform	Uniform
Fruit attractiveness	Intermediate	Good	Intermediate	Good
Fruit quality	Good	medium	Good	medium
Spine texture	Stiff	Stiff	Stiff	Stiff
Spine density	Dense	Dense	medium	Dense
Spine colour	Dark red	Dark red	Dark red	red
Spine strength	Strong	medium	Strong	Strong
Aril colour	c-white	c-white	c-white	c-white
Aril thickness	medium	medium	medium	medium
Aril texture	Firm	Soft	Soft	Soft
Aril taste	Acid- Sweet	Acid- Sweet	Sweet	Sweet
Aril flavour	Intermediate	Intermediate	Strong	Strong
Aril juiciness	Juicy	Juicy	Juicy	Juicy
Attachment of aril to seed	medium	Good	medium	Good
Adherence of testa to aril	Tight	Poor	Tight	Tight

Note: ob=oblong, ov=ovoid, glob=globose, Unif=uniform, fr=ruit, co=colour

Fruit morphologies are presented in Figure1, showing the three fruit shapes observed: globose, ovoid, and oblong. Spine density fell into category dense and very dense. From the qualitative characteristics assessed by the panel is, sweet taste and easily separated aril from the seed were the favourite characteristics of pulasan fruit.

Fruit morphology (Fruit shape, Spine density and Aril colour)		
1. Fruit shape		
Globose 	Ovoid 	oblong 
2. Spine density		
Sparse 	Medium 	Dense 
3. Aril colour		
white 	Dull white 	Cream white 

Fig. 1. Fruit morphology seen from 1). fruit shape (Globose, ovoid, oblong), 2). Spin density (Sparse, Medium, Dense) 3). Aril color (white, white-dull, cream-white)

Qualitative Characteristics Observed

Pulasan fruit from four locations in Riau Daratan had the same shape, which was ovoid. The fruit shapes of pulasan from Riau Kepulauan were different between the two locations. Fruit from Siak Regency was mainly globose, while fruit from Bengkalis oblong.

Grouping Among Populations in Riau Daratan and Riau Kepulauan

Based on dendrogram UPGMA (Figure 2) at coefficient similarity 92% it is seen that pulasan within Riau Regencies divided into two main group. The first group consisted of genotypes from Kampar kiri, Gunung Sahilan and Kuok, and the second group consisted of those from Kuantan Singingi, Bengkalis and Siak. All of the genotypes within the first group originated from Kampar Regency which is the area of Riau Daratan. Interestingly, the second group was originated from Riau Daratan and also Riau Kepulauan, in which genotype from Kuantan Singingi belonged to group of Riau Kepulauan. In Riau Daratan pulasan accessed were grown in mineral soil, while from Riau Kepulauan the populations were obtained from pulasan grow in peat soil (Fig. 3).

At coefficient similarity 73%, all the pulasan within Riau Provinces were the same.

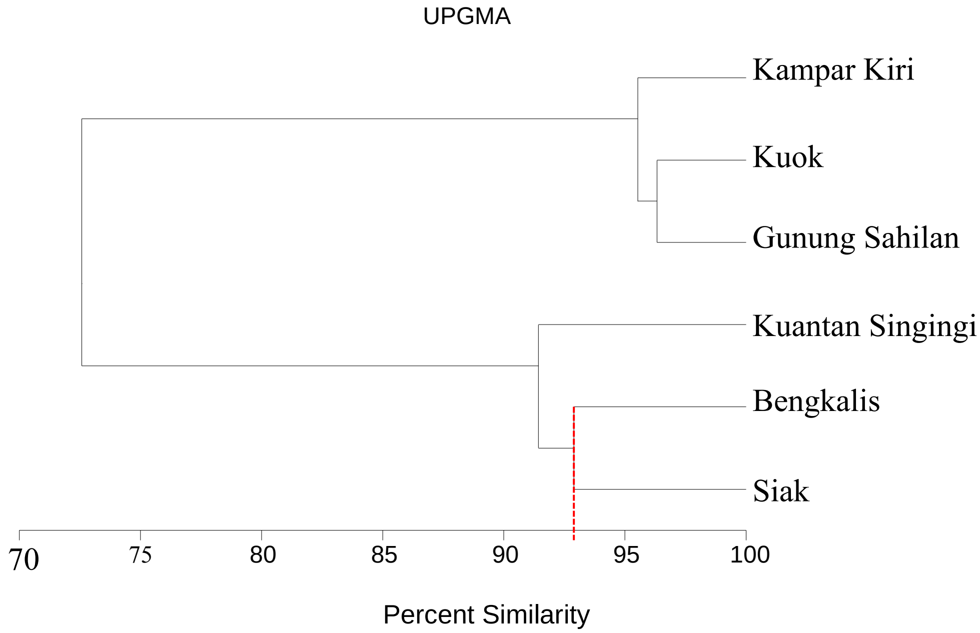

Fig. 2. Similarity percentage of pulasan originated from Riau Daratan and Riau Kepulauan

Pulasan as native plant of Indonesia is a close relative of rambutan. However, the genetic diversity of pulasan is not as vast as rambutan whereas the latest is easily found in tropical area like Indonesia. Pulasans are found in clusters in certain area, where rambutan also found.

However, Pulasan is not always found where rambutan occurs. The previous information reported that pulasan were found in West Kalimantan (Uji, 2007; Antarlina 2010), West Java (*Pamungkas 2015*) and West Sumatera (*Ediwirman et al*., *(2011*). This study adds information regarding the distribution of Pulasan in Indonesia, where pulasan were found in mineral and peat soil, even in saline area in the shoreline.

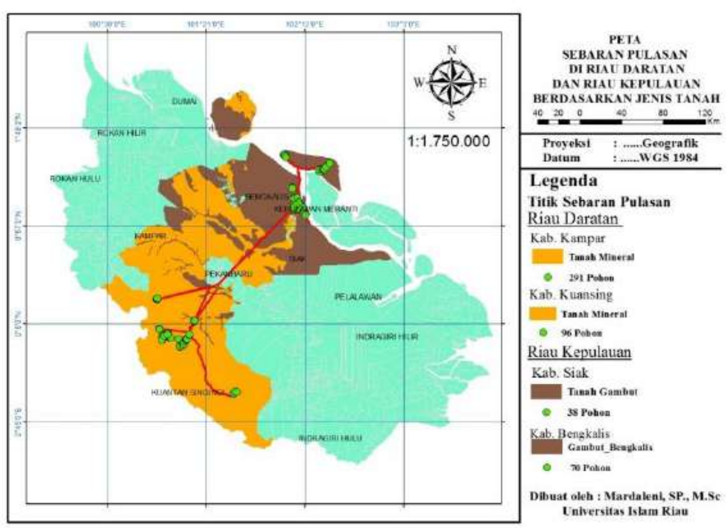

Fig. 3. Riau Provinces showing distribution of pulasan in different soil types

Conclusions

Fruit morphology from 65 pulasan accessions originated from Riau Daratan and Riau Kepulauan showed similarity at 73% coefficient similarity. Within Riau Daratan, the genotype similarity among population reached 96%, while within populations from Riau Kepulauanwas 93%, However variation within population was very high. Pulasan from Riau Daratan possessed edible part (aril) higher than that of from Riau Kepulauan. Fruit sweetness, obtained through ratio of total dissolved solid over total dissolved acid indicated that both group of pulasan fruit was sweet, however, pulasan fruit from Riau Kepulauan had higher ratio than that of Riau Daratan.

Sweetness and adherence characteristics of aril are the favourite character own by pulasan liked by panelis.

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Optimizing the Application of Calcium Ascorbate and Calcium Lactate for Increased Antimicrobial Efficacy on Fresh-cut Jackfruit (*Artocarpus Heterophyllus*)

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Abstract

Public awareness of health causes the preference of fruit and vegetable consumers require minimally processed foods with high nutritional value, fresh sensory attributes and lack of preservatives. This causes the demand and industries of fresh-cut fruits and vegetables are developed and increased. This study was conducted to determine the antimicrobial efficacy of calcium ascorbate and calcium lactate and evaluate the physicochemical and sensory properties of fresh-cut jackfruit. Calcium ascorbate and calcium lactate were dipped for 2 minutes at 1.5-2.5% concentration, with 150 ppm chlorine and distilled water as controls. The treated product samples were packed in sterile resealable 50 µm-thick polyethylene bag and stored at 7-10 ^{o}C for 5 days.

Calcium ascorbate and calcium lactate significantly decreased coliform and aerobic bacteria load and yeast and molds population on both fresh-cut products, with 2.5% concentration being sufficient. The physicochemical and sensory properties of the fresh-cut products after five days of cold storage were not adversely affected by the treatments.

Keywords: Artocarpus heterophyllus*, jackfruit, organic salts, calcium ascorbate, calcium lactate*

Introduction

Jackfruit (*Artocarpus heterophyllus* L.) is also known for its versatility of uses, providing food, timber, fuel, industrial and medicinal products. Jackfruit's primary economic produce is the fruit, utilized both when immature and mature. Grown in most tropical climates and is considered an exotic multiple fruit composed of hundreds of thousands of flowers having a weight reaching as much as 55 kg (120 lb). The yellowish avrils or bulbs constituting the perianth portion of the fruit are fleshy, fibrous, and rich in sugars as well as carotenoids. Each bulb has a single seed, which is edible after roasting. Rahman *et al*., [1] described the fruit as a rich source of carbohydrates, minerals, carboxylic acids, dietary fibre, and vitamins such as ascorbic acid (AA), and thiamine.

Generally, jackfruit is sold as a fresh-cut product mostly in open-air markets. Preparation of jackfruit is difficult and time-consuming because the latex exudes during cutting. They are either cut in half longitudinally or in cross-sections with the seeds removed. Most fresh-cut jackfruits are prepared by vendors in wet markets and along the roads, were fruitlets are displayed in bulk, or with ice during summer, and packaged only as needed by the consumer. Hence, causes degradation in quality caused by increased contamination through microbes, exhaustion of phytochemicals, tissue softening and browning.

Public awareness of health brings the preference of fresh-cut fruit and vegetable consumers require minimally processed foods with high nutritional value, with fresh sensory attributes and lack of preservatives. For this reason, the demand for minimal processing in industries of fresh-cut fruits and vegetables are developed and increased. The minimal processing of fruits and vegetables have acquired the swift trend among consumers because of its nutritional benefits, fresh like nature and ease in use. The processes like washing, cleaning, sorting, grading, peeling, cutting and slicing are some examples of minimal processing. However, according to Sexena *et al*., [2] minimal processing of pre- cut fruits and vegetables affect the quality by increasing the oxidative stress which tend to lose storing quality, not only in terms of microbial contamination, excessive softening, and browning, but also in terms of significant depletion of phytochemicals, such as phenolics, flavonoids, AA, and carotenoids. Cell disruption leads to release and intermixing of enzymes and substrates that may be used by native or exogenous microorganisms to grow on the product.

The infusion of additives during minimal processing has been described, to minimise these deteriorative changes in fresh-cut fruits and vegetables [3]. The application of natural treatments such as organic acids and Lactic Acid Bacteria on fresh-cut products coincide with the current trend on developing safe and sustainable antimicrobial systems [4]. In a previous study by Saxena *et al*., [5] presented that synergistic effect of anti-browning and antimicrobial compounds with reduced O_2 and elevated CO_2 atmosphere could enhance the quality of fresh-cut jackfruit avrils by minimising deteriorative changes in microbial attributes and physicochemical and sensorial qualities. Saxena *et al*., [6] have also presented the stabilisation of high moisture of jackfruit slices by a multi-target preservation technique.

Many available citations illustrated the advantages of numerous processing techniques in retaining the quality of minimally processed fruits and vegetables ([3], [7], [8]). Techniques like irradiation assisted with dip treatment chemicals like potassium meta-bisulphite and citric acid were reported favourable in retention of quality in minimal processed vegetables [9]. The use of anti-browning chemical such as ascorbic acid and citric acid prevent enzymatic browning in vegetables and fruits [10]. A study by Piga *et al*., [11] on the storage of minimally processed fruits and vegetables at low temperature (4 to 6 ^{o}C) reported extension in their shelf life. According to Koukounaras *et al*., [8] browning, tissue softening and weight loss are major problems in storage of minimally processed products. The treatment of coating with sucrose, trehalose and NaCl on minimally processed apples reportedly solved those problems [7]. Dip-treatment solo or in amalgamation with further techniques results advantageous in terms of maintaining colour and texture, prevent browning, extend shelf life and improves sensory parameters of product [3].

Neetoo *et al*., [12] reposted that lactic acid was not effective in meat and fish products, but, lactic acid (2.5-5% as 1-2 min dip) and its salts (5-10% sodium lactate as 1-2 min dip) were effective against *L. monocytogenes*. In mango cubes, the antioxidant ascorbic acid (1.5%) reduced microbial growth and doubled shelf life at 4 °C [13].

Treatment optimization for minimal processing of fruits and vegetables to maintain product's quality could results in increase of commercial market of minimal processed products. Hence, the need to develop a technology or process operations for fresh-cut jackfruit in dealing with the problems such as browning, colour loss, flavour loss, decaying and tissue softening. The optimization of minimal processing parameter of jackfruit will be fruitful to overcome the problems of marketing, handling and transportation. Thus, this study aims to determine the antimicrobial efficacy of calcium ascorbate and calcium lactate on fresh-cut jackfruit and to evaluate its physicochemical and sensory qualities of the treated and untreated fresh-cut jackfruit.

Materials and Methods

Whole ripe jackfruit was sliced, the arils were extracted, and the seeds removed under sanitary conditions. At least 200 g samples were used for each treatment per replicate. Two concentrations (1.5 and 2.5%) of two organic salts (Calcium ascorbate and calcium lactate) were tested, with 150 ppm sodium hypochlorite and distilled water where used as controls, replicated three times and applied as 2 min immersion. Samples were then packed in sterile, resealable 50 µm-thick polyethylene bags at 7-10 °C. Microbial enumeration following the [14] methods; plate count agar (total aerobic bacteria), violet red bile agar (coliform), and potato dextrose agar (yeast and moulds) were done after 3 h from treatment and after five days of storage under ambient conditions (27-33 °C). Product quality was evaluated in terms of the physicochemical qualities; colour using a Minolta CR-13 colorimeter, total soluble solids (TSS) using an Atago N1 Hand Refractometer, pH using an Orion pH meter model 210 A+, titratable acidity (% citric acid) by titration using standard 0.1 N NaOH and 1% phenolphthalein indicator, and for sensory qualities (colour, aroma, taste, texture, and aftertaste) using 15 trained panellists and 1-5 rating scale describing the variation in each attribute and 9-point Hedonic scale for acceptability of each attribute; the general acceptability was also evaluated using a 9-point Hedonic scale. Statistical analysis of results used the CROPSTAT version 7.2 program of IRRI.

Results and Discussion

Microbial load

Calcium ascorbate and calcium lactate were consistently effective at 1.5-2.5% in lowering aerobic, coliform, and yeast and molds counts after 3 hours from treatment and at 2.5% after 5 days of storage (Fig. 1, 2; Table 1). Chlorine had comparable antimicrobial effect only after 3 hours from treatment and was ineffective after 5 days of storage. Aerobic contaminants were mostly Gram-negative cocci and few short rods; coliform contaminants were mostly Gram-negative short rods; yeast and molds contaminants were greenish and brownish colonies (*Penicllium sp. & Aspegillus sp.*) and white colonies of yeast (*Saccharomyces*). Recently, the antimicrobial property of calcium lactate has been shown to be greater than that of sodium lactate and potassium lactate ([15], [16], [17]). On the other hand, calcium ascorbate could enhance resistance to microbial infection by stabilizing or strengthening cell walls [18].

After 3 hours from treatment **After 5 days storage at 7-10ºC**

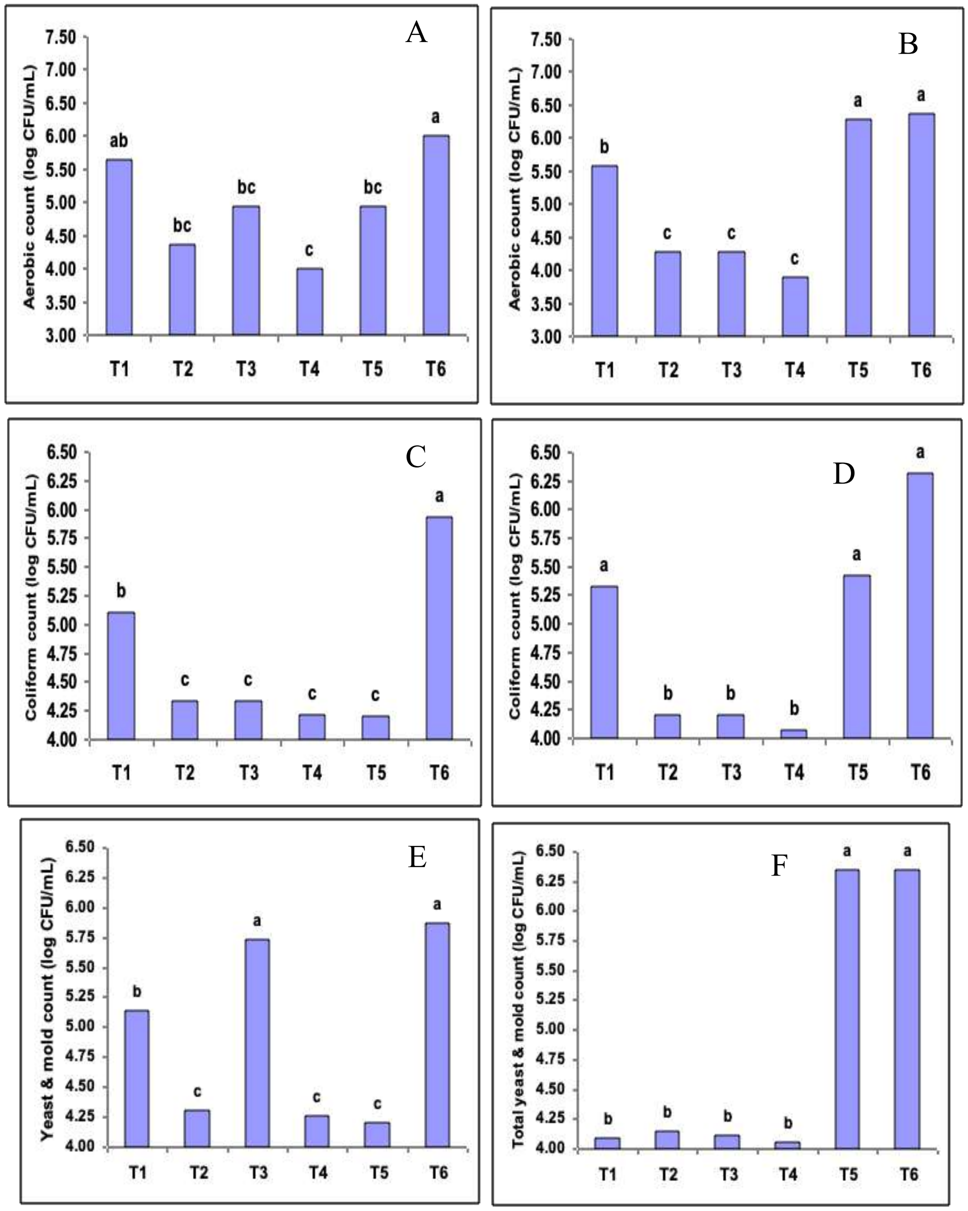

Fig. 1. Microbial load on organic salt-treated and untreated fresh-cut jackfruit.
Note: T_1-Ca ascorbate 1.5%; T_2-Ca ascorbate 2.5%; T_3-Ca lactate 1.5%; T_4- Ca lactate 2.5%; T_5-Chlorine; T_6-Water. Treatment means w/ same letter are not significantly different using LSD-5%, % CV: A-11.6; B-7.4; C-7.6; D-11.6; E-5.4; F-2.8)

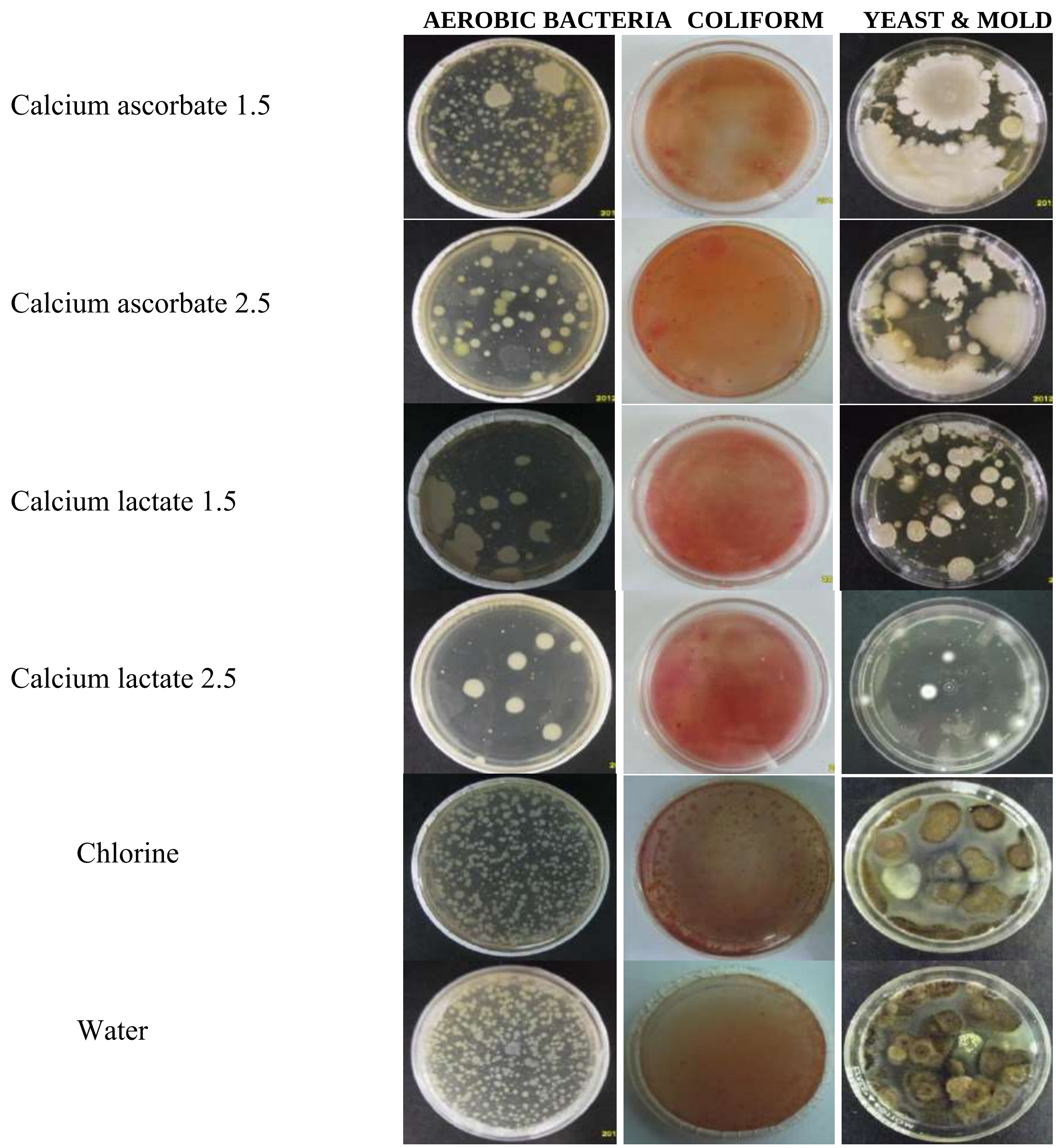

Fig. 2. Aerobic & coliform bacteria and yeast & mold colonies from cultured sample 10^{-4} dilution from organic salt-treated and untreated fresh-cut jackfruit after 5 days storage at 7-10 ^{o}C

Table 1. Percent reduction of microbial load on organic salt-treated fresh-cut jackfruit after 3 hours from treatment and after 5 days storage at 7-10 ºC

TREATMENTS	TOTAL AEROBIC COUNT		COLIFORM COUNT		YEAST & MOLD COUNT	
	(% reduction)		(% reduction)		(% reduction)	
	After 3 h	After 5 d	After 3 h	After 5 d	After 3 h	After 5 d
Relative to Water Wash						
Calcium ascorbate 1.5%	*	64.6	50.5	69.2	69.8	99.5
Calcium ascorbate 2.5%	97.8	99.2	97.5	99.2	97.2	99.4
Calcium lactate 1.5%	81.6	99.2	97.5	99.2	26.7	99.4
Calcium lactate 2.5%	99.0	99.6	98.1	99.4	97.5	99.5
Relative to Chlorine						
Calcium ascorbate 1.5%	*	57.7	*	*	*	99.5
Calcium ascorbate 2.5%	86.2	99.0	*	97.2	*	99.4
Calcium lactate 1.5%	*	99.0	*	97.2	*	99.4
Calcium lactate 2.5%	93.8	99.6	*	97.9	*	99.4

Percent reduction was calculated using actual microbial counts. ns – microbial count not significantly different from that of no wash or water wash. * – Microbial counts were similar or higher than that of water or chlorine treatment.

The inhibitory effect of calcium salts on microbial growth has been related to cell wall stability by ionic calcium and polygalacturonic chains, since calcium increases the rigidity of the cell wall and middle lamella and therefore the resistance to microbial enzymes responsible for cell wall degradation and tissue softening [19]. Antimicrobial properties of calcium lactate have been reported by Gorny *et al*., [20]. Calcium lactate was tested on fresh-cut lettuce and carrots and compared with chlorine. As alternative to chlorine, calcium lactate showed no differences in affecting the quality of the product, and both treatments showed similar effectiveness in reducing the microbial load. Calcium lactate is a form of calcium that is produced when lactic acid reacts with calcium carbonate.

Similar to calcium lactate, calcium ascorbate was found to have the same effect as chlorine in reducing microbial load [21]. It was revealed that calcium ascorbate reduced microbial counts at a pH range of 3-7 whereas the effect of calcium lactate was pH-dependent, indicating that the anion is responsible for the antimicrobial activity of calcium salts. An interaction between pH and calcium salt for antimicrobial activity is expected when the salt is derived from an organic acid, given the effect of pH on the dissociation of acids. Calcium ascorbate is commonly used as an antioxidant, preservative and source of Vitamin C in foods. In a study of Caballero [22], calcium ascorbate was sprayed on fresh-cut fruits and vegetables to increase shelf-life and was found to be especially effective at preventing apples from turning brown. The inhibition of aerobic bacteria and coliforms was triggered by some important factors; one factor was the reduction of pH. The inhibitory effect depends on the type of acid and the concentration. Gram negative bacteria such as *Pseudomonas* and *Acinetobacter* were inhibited at pH<5.3 while Gram positive bacteria can be only inhibited at pH<4.

Product Quality

b* values did not statistically differ with treatments and ranged from 15.30-20.93 (Table 2). L* values differed as only 1.5% calcium lactate maintained the pre-storage value while the rest had significantly reduced L*. Pre-storage TSS value was maintained only in samples treated with 1.5-2.5% calcium ascorbate while in the other treatments, TSS increased significantly after 5 days of storage. Increases in pH were also noted in chlorine and water treatments while samples treated with calcium ascorbate or calcium lactate had lower or comparable values as the pre-storage pH.

This was supported by the TA values which sharply increased after 5 days of storage.

Table 2. Colorimetric b* and L* values, total soluble solids (TSS), pH and titratable acidity (TA) of organic salts-treated and untreated fresh-cut jackfruit before and after 5 days storage at 7-10 ºC

TREATMENTS	b*	L* (lightness)	TSS (ºBrix)	pH	TA (%)
Calcium ascorbate 1.5%	20.77	58.87b	14.47c	4.62d	1.06a
Calcium ascorbate 2.5%	17.93	58.33b	15.20c	4.63d	1.09a
Calcium lactate 1.5%	16.37	64.43a	16.27b	4.63d	0.92b
Calcium lactate 2.5%	16.57	57.03b	17.00ab	4.64cd	0.72bc
Chlorine	16.50	56.53b	17.13a	4.76b	0.53d
Distilled water	15.30	56.73b	17.40a	4.87a	0.54d
Before storage (initial)	20.93	64.47a	15.10c	4.66c	0.21e

b indicates green (lower values) or yellow (higher values). Treatment means with a common letter are not significantly different at 0.05 level LSD.*

Increases in TSS and TA are associated with advancing ripening marked by the accumulation of sugars and partial breakdown of some sugars to organic acids which can occur during cold storage ([23], [24], [25]). However, the increase in TA did not parallel the changes in pH. pH even increased in chlorine and water treatments, similar to that obtained in the preceding experiments, which may indicate more advanced tissue senescence. In terms of sensory quality, calcium ascorbate generally improved colour, aroma, taste and texture better than calcium lactate but the overall acceptability was scored higher for the latter and for the former (Table 3). Chlorine and water treatments were generally to have least preferred sensory traits among treatments. The results indicate that organic salt treatment had no adverse effect on the sensory qualities of fresh-cut jackfruit after 5 days storage.

Table 3. Descriptive (Des.) and acceptability (Acc.) ratings of sensory quality attributes and general acceptability of organic salt-treated and untreated fresh-cut jackfruit after 5 days storage at 7-10 ºC

TREATMENTS	COLOR		AROMA		TASTE		TEXTURE		AFTER-TASTE		GENERAL ACCEPTABILITY
	Des.	Acc.	Des.	Acc.	Des.	Acc.	Des.	Acc.	Des.	Acc.	
Ca ascorbate 1.5%	3.23 a	6.96 a	3.93 a	6.80 a	3.03ab	6.16a	3.00	5.03	3.17	5.56	6.83b
Ca ascorbate 2.5%	3.23 a	7.00 a	3.96 a	6.80 a	3.10a	6.03ab	3.10	5.16	3.10	5.56	6.83b
Ca lactate 1.5%	3.23 a	5.96 b	3.50 bc	6.03 b	2.93bc	6.00ab	3.06	5.16	3.10	5.50	7.16a
Ca lactate 2.5%	3.23 a	6.10 b	3.60 b	6.16 b	3.00ab	6.00ab	3.16	5.10	3.10	5.50	7.23a
Chlorine	2.83 b	5.26 c	3.30 c	5.73 b	2.83cd	5.90b	3.03	5.13	3.03	5.36	6.66bc
Water	2.83 b	5.10 c	2.43 d	4.60 c	2.76d	5.56c	2.96	4.97	3.00	5.33	6.63c

Colour descriptive rating: 5-yellow, 4-light yellow, 3-slight brownish yellow, 2-moderate brownish yellow, 1-intense brownish yellow.
Aroma descriptive rating: 5-strong jackfruit aroma, 4-moderate, 3-slight, 2-none or absent, 1-others, specify.
Taste descriptive rating: 5-extremely sweet, 4-very sweet, 3-sweet, 2-slightly sweet, 1-bland
Texture descriptive rating: 5-firm and crunchy, 4-moderately firm, 3-slightly firm, 2-soft, 1-very soft
Aftertaste descriptive rating: 5-very perceptible aftertaste, 4-perceptible, 3-moderately perceptible, 2-slightly perceptible, 1-none
Acceptability/General Acceptability rating: 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely

Conclusion

Calcium ascorbate and calcium lactate markedly reduced microbial population of fresh-cut jackfruit after 3 hrs and 5 days of storage at 7-10 °C. The results obtained in this study demonstrated that the use of these organic salts at 2.5% concentration was needed to elicit the effect, having similar results to those of washing fresh-cut pineapple with chlorine after 3 hrs from storage and showed significant result even after 5 days from storage. Hence, this organic salt treatment does seem to be a promising alternative for improving decontamination efficiency in the sanitation of fresh-cut jackfruit.

In addition, these decontamination techniques have shown an indirect detrimental effect on fresh-cut product quality by affecting plant tissue physiology and structure. For the sensory attributes, among salts, it was the calcium ascorbate treated fresh-cut jackfruit showed no adverse effects, while, others showed poor sensory quality after 5 days of storage.

In general, applying calcium ascorbate and calcium lactate at concentration of 2.5% ensured good quality of fresh-cut jackfruit, by reducing microbial load while maintaining good sensory acceptability of fresh-cut jackfruit even prolonging storage-life up to 5 days under cold storage.

Further research should focus on the optimization of the use of these organic salts in the production process of fresh-cut jackfruit.

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Development of Durian Breeding Population for Molecular Assisted Breeding

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Abstract

Durian (*Durio sp*.) breeding activity requires molecular-assisted selection strategy to obtain faster results. This research was aimed to develop durian breeding populations through intra and inter-species crosses among resistant, tolerant and susceptible parent trees for supporting molecular assisted breeding. The research was conducted at Indonesia Tropical Fruit Research Institute from November 2012 to September 2013. Breeding population was formed through reciprocal artificial cross pollination among six durian varieties: Chanee, Matahari, Monthong & Petruk (*D. zibethinus*), Lai Mas (*D. cannotus*), and Lai Mahakam (*D. kutejensis*). Artificial pollination was conducted from 19:00 to 23:00 pm. The fruit was harvested upon mature at 110-135 days after pollination, then the seeds were processed and sown in polybags containing mixed soil and compost media. Cross pollination among 6 durian parental trees have been carried out for 14 times involved 272 flowers which resulted 14 fruits (5.1%) and produced 154 seeds. Of these, 65 seeds were growing normally (42.21%), 44 seeds were abnormal (28.57%) and 45 seeds were dead (29.22%). Based on the number of progenies produced, the combination that meet the requirements to be used as mapping populations are crosses between Matahari x Chanee which produced total of 52 progenies.

Keywords: durian, breeding, population, MAS

Introduction

Durian superior variety could be obtained through selection of indigenous germplasm [1] or breeding program [2], [3], [4]. Structured breeding program is required to create ideal durian varieties and meet market demand in the future. In order to obtain faster results, the breeding activity requires to use strategies and techniques of molecular or genetic markers which known as marker-assisted selection (MAS) or molecular breeding [5]. The basic principle of this technology is to map or detect genetic markers associated with quantitative traits of interest (quantitave traits loci-QTL) which are inherited freely [6]. Mapping of genetic markers has developed and is widely used for breeding activities in the world's major food crops such as rice [7], maize [8], cassava [9], wheat [10], wheat [11], soybeans [12], soybean [13], oil palm [14], and citrus [15].

Mapping of genetic markers is based on Mendel's findings about inheriting freely segregated traits from predictable phenotypic characters [16]. Molecular markers are like "road signs" on chromosomes to help genome analysis in identifying DNA sequence locations or certain genes that are passed down to their progenies through the inheritance law from one generation to the off-springs [5], [17]. Molecular markers work based on DNA sequence polymorphisms due to deletion, substitution or insertion [16]. A genetic marker if it has been found can be used directly in breeding programs and makes it easier for breeders to predict phenotypic characters based on the presence/absence of these markers [5], [17].

In this research, MAS was applied to help mapping genetic markers associated with durian resistance to die-back diseases caused by Pythiaceae [18], [19]. This application is based on the assumption that the nature of resistance to pathogenic infections is determined by the presence of 'resistance genes' in the host [20]. A resistant host means that it contains genes that control the resistant properties in the chromosome. This gene is passed to its progeny through chromosomal recombination (segregation) during the meiosis process [21].

The MAS application starts with constructing the linkage group (LG) molecular markers. The three main steps in LG construction are mapping population construction, polymorphisms identification, and linkage marker analysis [5]. Mapping populations are generally derived from segregating plant population [22] or progeny obtained from advanced generation crosses such as F2, RIL, DH, NIL, BC1 [16], [23], or pseudo-test cross [24].

Objective of the study was to develop durian breeding populations through intra and inter-species crosses among resistant, tolerant and susceptible parent trees for supporting molecular assisted breeding.

Materials and Methods

Plant materials for constructing mapping populations were six durian varieties: Chanee, Lai Mas, Matahari, Monthong, Petruk, and Lai Mahakam. The characteristics of the six parent trees are listed in Table 1. Mapping population was constructed through reciprocal artificial cross pollinations among the six durian varieties. The flowers of female parent trees were castrated a time upon before blooming. Thinning was done on big cluster flowers to left 1 flower in the middle position, then the flower was emasculated by cutting all the stamens. Finally, the flowers were wrapped using paper pack. This work was carried out during 02:00 to 04:00 pm. The flowers of male parent were cut and put upright in a glass half-filled distilled water to allow to develop and bloom completely when artificial pollination was conducted at 07:00 to 11:00 pm. following the receptive time of the pistil. Pollination was carried out by directly attaching the pollens from the stamen of male flower to the stigma on the castrated female flower. The pollinated flowers were rewrapped with paper pack to avoid open pollination. Finally, the pollinated flowers were labelled for dates, numbers and combinations of females/males. Illustration of the artificial pollination is shown in Figure 1.

Castration at 2.00-4.00 pm | Flower was wrapped after castration | Artificial pollination at 07.00-11.00 pm | Immature fruit resulted of artificial pollination

Fig. 1. Illustration of artificial cross pollination for constructing durian mapping population

Four to five days after pollination, the wrap was opened and replaced by a net to reduce damage to the immature fruit. At 110-120 days after pollination, the fruit was harvested and the seeds were proceeding by separated from the aril by washing with fresh water. The seeds were then sown in polybags containing mix soil and compost media.

Results and Discussion

The availability of mapping populations originating from biparental crosses is the first requirement for constructing a linkage map [22]. Artificial pollinations to provide population mapping have been carried out by incomplete reciprocal artificial pollination among 6 durian parent trees. A total of 272 flowers were pollinated which resulted in 14 (5.1%) fruits and produced 144 seeds, where 65 progenies were grew normal, 44 progenies were abnormal and 45 progenies were dead. In general, abnormal plants showed slower growth and many were rosettes. Many dead plants preceded with abnormal symptoms. The parent combination data and the crossing results are shown in Table 2.

Amongst all crosses combinations made, reciprocal crosses were only carried out between Matahari and Lai Mahakam parent trees for 3 times with covered a total of 148 flowers and succeeded in producing progeny. Two of the three pollinations found to produce fruit, while one fruit were fell out at about 30 days after pollination. Four of one-way crosses combinations also succeeded in producing progeny were Matahari x Petruk, Matahari x Chanee, Matahari x Monthong, and Lai Mas x Petruk.

Crosses between Matahari and Lai Mahakam produced 3 fruits which resulted 14 progenies, where 3 progenies were normal, 3 progenies were abnormal, and 8 progenies were dead. Crosses between Matahari and Petruk produced 1 fruit which resulted 19 progenies where 18 progenies were normal and 1 progeny was dead. Crosses between Matahari x Chanee produced 4 fruits which resulted 84 progenies, where 35 progenies were normal, 17 progenies were progenies were abnormal and 11 progenies were dead. Crosses between Matahari and Montong Dpj produced 6 fruits which resulted 50 progenies, where 4 progenies were normal, 21 progenies were abnormal, and 25 progenies were dead. Crosses between Lai Mas and Petruk produced one fruit which resulted 10 progenies, where 5 progenies were normal and 5 progenies were abnormal.

Successfully artificial pollinations found to produce fruit sets vary from 0-22.22% with an average of 5.1%. This value was higher than natural fruit set, which averages between 0-1.56% [25], [26], but lower than the results of the artificial pollination conducted by Honso [25] which reached 27.2%. Besides fruit set which was not high enough, pollination activities cannot be carried out at all combinations reciprocally because of several obstacles faced, including: (i) parent trees are far apart each other, (ii) flower maturing do not at the same time, (iii) parent tree is in low health level, (iv) heavy rain during pollination that causes the flowers to fall out, and (v) the status of nutrients, so that the fruit will not be able to reach physiological cooking age.

Problems in providing mapping population were not only during pollination, but also during seeding maintenance. The progenies turned to be abnormal and dead during maintaining in the nursery. From 154 seed, progenies with normal growth were only 65 plants (42.21%), while 44 (28.57%) were abnormal and 45 (29.22) dead. In general, abnormal plants show slow growth and many also have rosettes (branching a lot and short), as well as dead plants that generally preceded with rosette.

From this crossing only Matahari x Chanee which produces quite a lot of progeny, namely 52 plants. This amount fulfils the requirements to be used as a mapping population. According to Collard [5] for the initial stage of the preparation of linkage groups requires a population of 50-250 progenies in order to get good recombination.

Conclusion

Reciprocal crosses between 6 durians involving 272 flowers produced 14 (5.1%) fruits and resulted 154 progenies, where 65 (42.21%) progenies were normal, 44 (28.57%) were abnormal and 45 (29.22) dead. Based on the progeny number produced, the combination that meet the requirements to be used as mapping populations are crosses between Matahari and Chanee which produced total of 52 progenies.

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Table 1. Characteristic of 6 parent trees to construct F1 durian breeding population

No	Character	Chanee	Lai Mas	Matahari	Monthong	Petruk	Lai Mahakam
1.	Tree size	big	medium	big	big	medium	medium
2.	Leaf size	medium	wide	medium	medium	medium	extra wide
3.	Odour	strong	weak	strong	weak	strong	weak
4.	Fruit size	big	medium	big	extra big	medium	medium
5.	Fruit shape	rounded	oblong	rounded	irregular	rounded	rounded
6.	Fruit colour	green-brown	yellow-brown	green-brown	green-brown	green-brown	yellow-brown
7.	Aril colour	yellow-orange	orange	yellow	light yellow	light yellow	orange-red
8.	Aril thickness	medium	medium	medium	thick	medium	medium
9.	Seed mature	mature	mature	mature	immature	mature	partly immature
10.	Aril firmness	less firm	firm	firm	less firm	firm	firm
11.	Taste	sweet	sweet	sweet-bitter	sweet	sweet-bitter	sweet
12.	Self-life	long	long	medium	long	short	long
13.	Disease resistant	resistant	resistant	tolerant	susceptible	susceptible	susceptible
14.	Finesse	medium	early	medium	early	medium	early
15.	Productivity	high	medium	medium	high	medium	medium

Table 2. Artificial pollination of 6 parent trees to develop breeding population

No	Parent combination	No of flower	No of fruit	Fruit set (%)	No of seed	Remark
1.	Matahari x Petruk	10	1	10	19	normal= 18; mati= 1
2.	Petruk x Matahari	3	0	0	0	fall at anthesis; illness tree
3.	Matahari x Chanee	10	1	10	19	normal= 11; dead= 8
4.	Matahari x Chanee	25	3	0,12	44	normal= 24; abnormal= 17; dead= 3
5.	Chanee x Matahari	25	0	0	0	fall at anthesis
6.	Matahari x Montong Dpj	25	6	0,24	50	normal= 4; abnormal= 21; dead= 25
7.	Matahari x Lai mas	25	0	0	0	fall at anthesis
8.	Matahari x Lai Mahakam	9	2	22,22	14	normal= 4; abnormal= 2; mati= 8
9.	Matahari x Lai Mahakam	27	0	0	0	fall at anthesis
10.	Matahari x Lai Mahakam	45	0	0	0	fall at immature fruit
11.	Lai Mahakam x Matahari	18	0	0	0	fall at anthesis
12.	Lai Mahakam x Matahari	30	0	0	0	fall at anthesis
13.	Lai Mahakam x Matahari	17	0	0	0	fall at immature fruit
14.	Lai Mas x Petruk	10	1	10	10	normal= 5; abnormal= 5
		272	14	5,1	154	Normal= 65; abnormal= 45; dead= 44

Leaf Sample for Nutrient Status Analysis on Lansium Fruit Tree

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Abstract

Chemical analysis of the leaves will reflect changes more accurately in plant nutrient status as impact of fertilization in production. The objectives of this research were to determine proper leaves as samples and to determine optimum N, P, K nutrients status of duku tree. The research was conducted in Pemunduran Village, Kumpeh Ulu District, Muaro Jambi Regency in Jambi Province. Chemical analysis was carried out at laboratory of Indonesian Soil Research Institute.

Twenty duku trees, with relatively same age (30-40 years) were used in this research. Mature leaves in the terminal branches were sampled and taken before harvest, at harvest and after harvest time. The leaves samples position was at the first and the third leaves from fruiting and non-fruiting branches. The results indicated that the third mature leaves in non-fruiting branches were given high correlation with relative yield (coefficient correlation 0.87, 0.74 and 0.71 for N, P, and K, respectively). The third or first mature leaves during harvest time of the fruiting branches could be used as alternative for diagnose N, P and K nutrient status of duku plants. Leaves nutrient concentrations were 1.74-2.43%, 0.19-0.25% and 1.85-2.77% for N, P, K, respectively.

Keywords: Lansium domesticum*, nutrient status, leaf analysis*

Introduction

Lansium or duku (*Lansium domesticum*) is native fruit of Indonesia. The fruit are marketed in traditional and modern market. Duku tree has deep and extensive rooting and normally do not fertilized or cultivated. Weeds and shrubs around the tree are rarely clean, only when duku begin to bear fruit. The traditional fruit tree management has caused low production of duku, where an optimum availability of mineral nutrients for maximum growth is not provided. Fertilization for perennial trees is very important to obtain high yield and good quality of horticulture crops [1], [2].

Leaf analysis to determine status of nutrients in the plant is a good method for assessing nutrient needs of perennial crops. In perennial plants, the prediction of the availability of mineral nutrients on the root depth effective for crop growth is difficult [3]. Leaf analysis is a method for estimating plant nutrient requirements based on the assumption of, "within certain limits, there is a positive inter relationship among the rate of nutrient supplied, leaf nutrient content and consequently yield and/or quality". Nutrient supply in one year may have a major effect on both fruit tree nutrition status and crop production in subsequent years, as the plant respond to direct and residual soil fertility [4], [5], [6], [7].

Nutrient analysis of leaves had been used extensively as a diagnostic tool for perennial estate crop to determine the nutrient needs for plants prior to produce on optimum yield. Leaf nutrient status can be used as an index to determine the nutrient status of the plant, which is associated with growth and production of plants [8], [9]. Leaf nutrient concentration is influenced by the position of leaves in the canopy. Alva *et al*., [10]; Razeto and Salgado [11]; Yen [12]; Zeng and Brown [13] stated that, N, P and K concentration had a greater variation among leaves on the non-fruiting branches. Concentrations of N, P and K in the leaves of fruit crops varied, among the leaf position in the canopy. The result of the study on mango, fifth leaf from base of current flush after harvest was the best leaf nutrient status determination [14]. Menzel *et al*., [15] have shown that the plant lychees for nutrient diagnose was flowering branches, i.e., 1-2 weeks after panicle appeared.

There are three types shoots in fruit crops, in the first group, the entire shoot emerges at one time and therefore, all the new leaves have the same age. In the second group, the shoot grows continuously and therefore, each leaf has a different age, and third, the shoot throws new growth as well as give away branches after every two leaves [4]. The shoot of duku is the first type, the entire shoot emerges at one time, and therefore, all the leaves have the same age. Proper leaf sampling can be implemented if the nutrient concentration changes during the period of development on the plant have the best correlation with the production [4].

Kidder [16] stated that, in order to obtain the best relationship between yield and the level of a substance in the leaves could be detected through correlation test. The leaves which are best correlated with the yield, then are used in the calibration test. Leaf analysis values obtained from the laboratory associated with the yield could be classified into: very low, low, medium, high, and very high of nutrient status.

In lansium plants, which leaves could best describe the nutrient status have not been known in Indonesian. When appropriate leaf samples have been known, then it could be used to determine the nutrient status categories and the corresponding models to predict the crop response to fertilizer application. Correlation between concentration of N, P and K in various leaves positions and yield of lansium could be established. The objectives of this research were to determine the proper leaves as sample for nutrient analysis to diagnosis and to determine optimum N, P, K nutrients status of lansium based on the leaves position.

Methodology

The research was conducted in area of lansium central production at Kumpeh Ulu District, in Jambi Province, 10 meters asl. Chemical analysis was carried out at laboratory of the Indonesian Soil Research Institute. Leaf samples from the lansium tree of Kumpeh variety (aged 30-40 years) as many as 20 trees. Lansium plant spacing was 10x10 m, soil type of alluvial and located in the watershed (DAS) Batanghari. Leaves samples were matured leaves in the terminal branches, i.e., mature leaves before fruit harvest time, mature leaves at fruit harvest time, and mature leaves after harvest. The leaf sample position was the first and the third leaves from fruiting and non-fruiting branches (Fig. 1 and Table 1).

The leaves taken for analysis were those exposed to the sun and located at the bottom of the canopy, taken at 07.00-09.00 am. Leaf samples were cleaned with a tissue, dried in an oven at a temperature of 90 °C for the first two hours and then at reaching 70 °C until constant weight. The dried leaves were blended and sieved with a 0.5 mm, then were analysed for their concentration of N, P and K. Analysis of total N used the Kjeldahl method, while P and K each were done with wet incineration. Measurement of N and P were performed with the ultraviolet visible spectrophotometer, while K with the atomic absorption spectrophotometer.

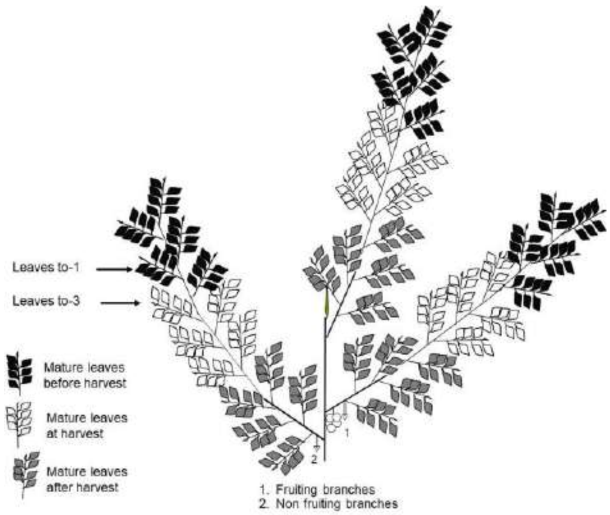

Fig. 1. The position of leaves samples on the leaf canopy

Soil samples from the root zone were taken at five core points at the experiment sites, and then composited into two groups, based on soil depth of 0-30 cm and 30-60 cm. The chemical analysis of the soil samples consisted of pH, CEC, organic-C, total N, potential and available of P and K, exchangeable Ca and Mg. Data were also collected for fruit yield/tree, ground water level and climate characteristic. Ground water level was observed at every two or three weeks, and climate observations consisted of temperature, humidity and rainfall during the research. Fruit yield/tree in the form of relative yield (% RY), was calculated by the following formula:

$$\text{Relative yield} = \frac{Yi}{Y_{max}} \times 100\%$$

Yi = fruit yield of i^{th} tree
Y_{max} = maximum fruit yield of 20 tree sample

The correlation between concentration of N, P and K in the leaves of each leaf position and relative yield of lansium tree were analysed with simple linear equation. Leaf concentrations of N, P and K which had high correlation values was in used the calibration test.

Table 1. Sampling time and leaf position of lansiun tree

Leaf position	Branch condition	Leaf condition	Sampling time
1^{st} leaves	Non fruiting	Mature	Six-month before harvest
1^{st} leaves	Fruiting	Mature	Six-month before harvest
3^{nd} leaves	Non fruiting	Mature	Six-month before harvest
3^{nd} leaves	Fruiting	Mature	Six-month before harvest
1^{st} leaves	Non fruiting	Mature	At harvest
1^{st} leaves	Fruiting	Mature	At harvest
3^{nd} leaves	Non fruiting	Mature	At harvest
3^{nd} leaves	Fruiting	Mature	At harvest
1^{st} leaves	Non fruiting	Mature	Six-month after harvest
1^{st} leaves	Fruiting	Mature	Six-month after harvest
3^{nd} leaves	Non fruiting	Mature	Six-month after harvest
3^{nd} leaves	Fruiting	Mature	Six-month after harvest

Results and Discussion

N, P and K Concentration and Positions of Duku Leaves

Concentration of N, P and K at various leaves positions showed different values. The concentration of the three nutrients at the terminal non fruiting branches was higher than those at the fruiting branches (Table 2-4).

The concentration N, P and K third mature leaves at harvest time of non-fruiting branches, had the highest correlation with relative yield, in the with a correlation coefficient 0.87 for N, 0.74 for P and 0.71 for K (Table 2, 3, 4). These leaves therefore, could be used to diagnose and determine the optimum of N, P and K nutrient status on lansium tree and will be used for calibration test.

Duku leaves are compound leaves with 5-7 leaflets and the third leaf is located in the middle.

Generally, the sixth and seventh leaf is damaged or not intact. The N, P and K nutrient concentrations in the third leaf are more stable and optimum compared to the compounded leaves.

Correlation coefficients of N, P and K with the fruit yield were lower in fruiting branches.

Table 2. Correlation coefficient between N concentration in various leaf positions and relative yield (RY) of lansium trees

Leaf position	N Concentration of leaf (%)	Correlation coefficient between N and RY
1st leaves before harvest, non-fruiting	1.80±0.35	0.55*
1st leaves before harvest, fruiting	1.56±0.32	0.50*
3rd leaves before harvest, non-fruiting	1.77±0.43	0.43
3rd leaves before harvest, fruiting	1.56±0.41	0.20
1st leaves at harvest, non-fruiting	1.67±0.19	0.74**
1st leaves at harvest, fruiting	**1.76±0.25**	**0.58****
3rd leaves at harvest, non-fruiting	**2.07±0.37**	**0.87****
3rd leaves at harvest, fruiting	**2.26±0.36**	**0.61****
1st leaves after harvest, non-fruiting	2.35±0.33	0.51*
1st leaves after harvest, fruiting	1.81±0.18	0.33
3rd leaves after harvest, non-fruiting	2.31±0.53	0.54*
3rd leaves after harvest, fruiting	1.89±0.19	0.11

**: significant at 5% probability level, **: significant at 1% probability level*

Table 3. Correlation coefficient between P concentration in various leaf positions and relative yield (RY) of lansium trees

Leaf position	**P Concentration of leaf (%)**	**Correlation coefficient between P and RY**
1st leaves before harvest, non-fruiting	0.09±0.04	0.59*
1st leaves before harvest, fruiting	0.10±0.06	0.20
3rd leaves before harvest, non-fruiting	0.10±0.05	0.55*
3rd leaves before harvest, fruiting	0.08±0.04	0.39
1st leaves at harvest, non-fruiting	0.19±0.02	0.55*
1st leaves at harvest, fruiting	**0.19±0.03**	**0.52***
3rd leaves at harvest, non-fruiting	**0.22± 0.04**	**0.74****
3rd leaves at harvest, fruiting	**0.22± 0.05**	**0.52***
1st leaves after harvest, non-fruiting	0.09±0.01	0.51*
1st leaves after harvest, fruiting	0.17±003	0.62**
3rd leaves after harvest, non-fruiting	0.10±0.02	0.59**
3rd leaves after harvest, fruiting	0.19±0.03	0.49

**: significant at 5% probability level **: significant at 1% probability level*

Table 4. Correlation coefficient between the K concentration at various leaf positions and relative yield (RY) of lansium trees

Leaves position	K Concentration of leaves (%)	Correlation coefficient between K and RY
1st leaves before harvest, non-fruiting	1.29±0.27	0.57*
1st leaves before harvest, fruiting	1.50±0.49	0.37
3rd leaves before harvest, non-fruiting	1.39±0.46	0.70**
3rd leaves before harvest, fruiting	1.19±0.24	0.51*
1st leaves at harvest, non-fruiting	1.77±0.45	0.50*
1st leaves at harvest, fruiting	**1.79±0.44**	**0.66****
3rd leaves at harvest, non-fruiting	**2.41±0.63**	**0.71****
3rd leaves at harvest, fruiting	**2.46±0.80**	**0.49***
1st leaves after harvest, non-fruiting	1.10±0.25	0.43
1st leaves after harvest, fruiting	1.92±0.26	0.53*
3rd leaves after harvest, non-fruiting	1.17± 0.19	0.37
3rd leaves after harvest, fruiting	2.20±0.34	0.21

**: significant at 5% probability level **: significant at 1% probability level*

The third matured leaf at harvest of non-fruiting branches of lansium could be used to determine the status of N, P, and K nutrients on the tree. The second alternative, if the entire branches bear fruits, the third or the first matured leaf at harvest could be used to diagnose the nutrient status of the tree (Table 2,3,4). Concentration of N in the third leaves position was better than others leaves position of the branches fruiting (Table 2), although the P and K values for the correlation coefficient were less than the first mature leaf after harvest, but it was practically more convenient to use the same leaf for analysis N, P and K. This is similar to Thamrin's research [17] pummelo citrus that the third-fourth leaf has the highest correlation with the yield. The third-fourth leaf after harvest on the previous fruit production, is best used to diagnose the N, P, and K nutrient status in Pangkep pamelo citrus because it contains N, P, and K nutrient concentrations and has a higher correlation with citrus fruit yield [17], [18].

Concentrations of N, P, K of Leaves and Relative Yield

The relationship between N, P and K concentration on leaves of the third matured leaves at harvest taken from non-fruiting branches and the relative yield of lansium tree in first and third year could be seen in Fig. 2. On the second year, lansium tree does not bear fruit. The first and second year is a condition of which the tree produces less yield, or known as the small harvest year (off year) and the third year the tree bear high fruit yield on known as the big harvest (on year).

Relationships between third matured leaves at harvest from the non-fruiting branches showed that r (coefficient correlation) value was the highest on third year harvest. Value of r on the big harvest was 0.87 for N, 0.74 for P, and 0.71 for K, while for the small harvest was 0.64 for N, 0.73 for P, and 0.77 for K. Determination the status of N, P and K in the leaves of lansium tree is more appropriate to be conducted during the big harvest. Leaves nutrients concentrations of N, P and K were shown on Table 5.

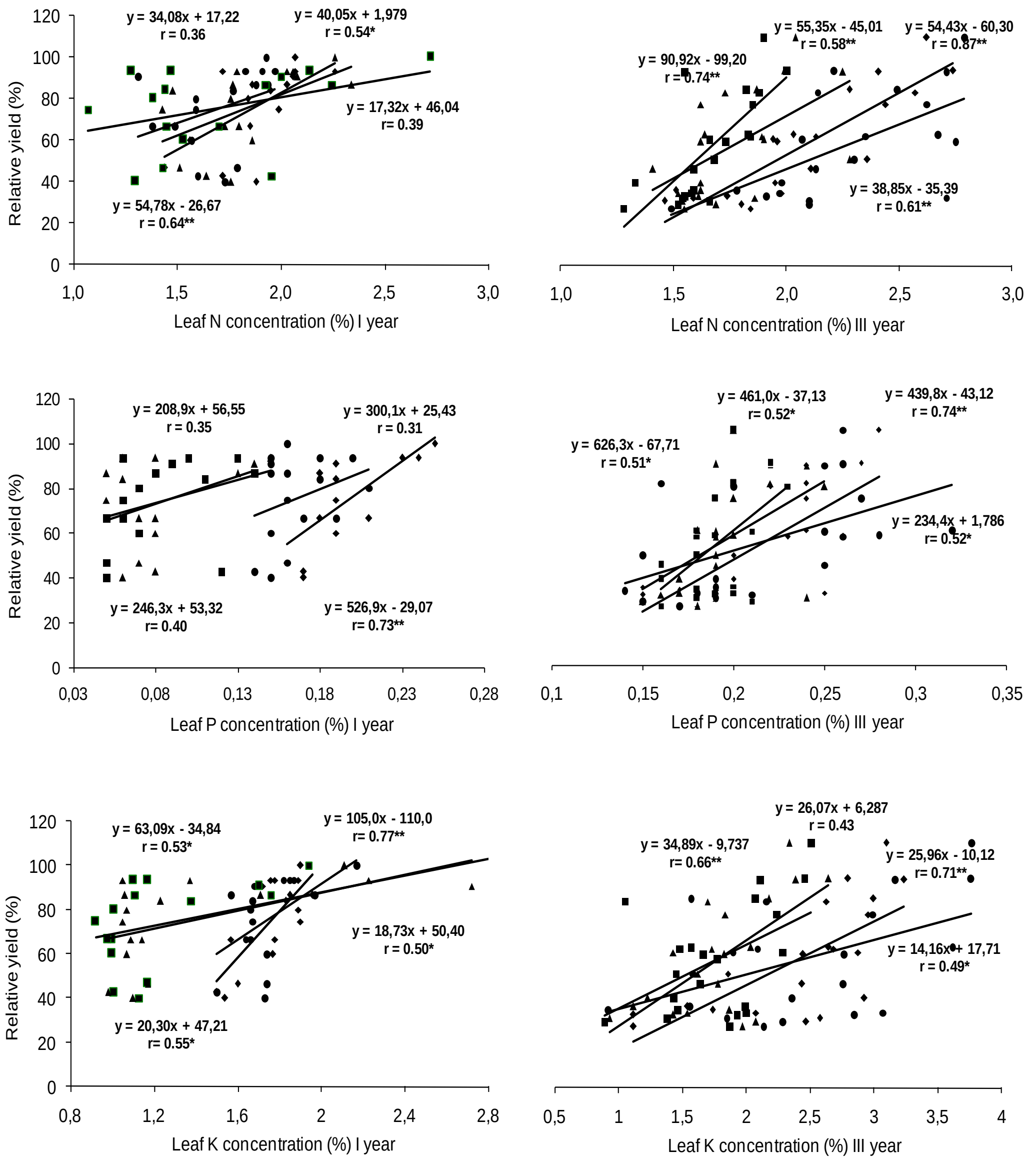

Fig. 2. The relationship between N, P and K concentration of the third mature leaves at harvest from branches non-fruiting and relative yield of duku I and III years.

Table 5. Lansium fruit yield, N, P dan K concentrations of leaves at during big harvest

N Concentration of leaves (%)	P Concentration of leaves (%)	K Concentration of leaves (%)	Fruit yield kg/tree	Relatif yield (%)
1.74±0.19	0.19±0.03	1.85±0.64	144.7±12.3	28.82+2.44
2.06±0.31	0.22±0.03	2.57±0.47	240.4±48.2	54.05+16.52
2.43±0.24	0.25±0.03	2.77±0.49	414.2±51.5	74.27+19.83

Variation of fruit yield among years were thought to be influenced by nutrient availability and climatic factors, especially rainfall. Rainfall in the study site was more fluctuating, while the temperature, humidity and soil water levels were relatively constant. Flowering and fruiting of lansium trees is affected by the length of dry months. Dry months is defined of fruiting season when rainfall is less than 60 mm/month [19]. In the first year move there were three dry months (April, August and September), where flowers and fruits formed in October, in a small amount.

The second year of fruiting season there were no dry months and there was no flowers and fruit formed in this year. The third year there were four dry months (February, July, September and December) and flower were formed in October.

Concentrations of N, P, K of Leaves and Soil Chemical Properties

Nutrients N, P, and K are the main nutrients used to increase the growth, production and quality of fruit [20]. Concentrations of N, P and K in the leaves was high when the fruit yield was high, it was apparent during in the third year of harvest (Figure 2), where the concentration of nutrients in the soil was lower (Table 5). Soil nitrogen at 0-30 cm depth was higher than that at 30-60 cm depth, namely 0,17 to 0,09 and 0.12% to 0.07%. Lansium is a perennial tree which has deep root.

Nutrients are absorbed by plants at the depth of 30-60 cm. This also occurs in other fruit plants, such as durian, nitrogen concentration in the leaf is positively correlated with the development of the reproductive period on the durian plant [21]. The available phosphorus in the soil also decreased in the third-year harvest, from 8.2 to 7.4 ppm, but at the depth of 30-60 cm P increased from 4.3 to 6.2 ppm. The concentration of P in the leaves almost the same in first- and third-year harvest. Highly significant decrease of fruit yield was seen in concentrations of soil K, from 0.24 to 0.07 (cmol (+)/kg) at a depth of 0-30 cm and 0.17 to 0.06 (cmol (+)/kg) at depth of 30-60 cm.

Potassium is an element that increase the fruit number and size, involves in the formation of carbohydrates, and sugar translocation in the xylem tissue formation [22], [23]. Thus, K in the soil will decrease with increasing fruit yield. Concentration of K tended to decrease during fruit development [24]. This shows that most nutrients in the tree come from the soil, and leaf nutrition was closely related to the soil nutrient [25], [26].

Table 6. The results of soil analysis in I to III years

Parameter	Horizon	Value			category*		
		I year	II years	III years	I year	II years	III years
pH (H_2O)	0-30 cm	4,5	4,3	4,0	Acid	Very acid	Very acid
	30-60 cm	4,6	4,4	4,0	Acid	Very acid	Very acid
C- organic (%)	0-30 cm	1,68	1,41	1,54	Low	Low	Low
	30-60 cm	0,88	0,77	0,85	Very low	Very low	Very low
total N (%)	0-30 cm	0,17	0,10	0,12	Low	Low	Low
	30-60 cm	0,09	0,07	0,07	Very low	Very low	Very low
P_2O_5 Bray I (ppm)	0-30 cm	8,2	6,8	7,4	Medium	Low	Low
	30-60 cm	4,3	4,0	6,2	Low	Very low	Low
P_2O_5 (HCl 25%)	0-30 cm	35	31	33	Medium	Medium	Medium
(mg/100g)	30-60 cm	34	31	29	Medium	Medium	Medium
K_2O HCl 25%	0-30 cm	30	15	14	Medium	Low	Low
(mg/100g)	30-60 cm	29	16	14	Medium	Low	Low
Mg ($cmol_{(+)}$/kg)	0-30 cm	2,47	2,19	2,73	High	High	High
	30-60 cm	2,15	1,80	2,19	High	High	High
Ca ($cmol_{(+)}$/kg)	0-30 cm	1,91	2,16	2,92	Very low	Low	Low
	30-60 cm	1,36	1,37	2,14	Very low	Very low	Low
K (cmol(+)/kg)	0-30 cm	0,24	0,10	0,07	Very low	Low	Low
	30-60 cm	0,17	0,08	0,06	Very low	Very low	Low
CEC ($cmol_{(+)}$/kg)	0-30 cm	14,42	9,61	9,63	Low	Low	Low
	30-60 cm	14,27	9,50	9,05	Low	Low	Low

** Source: Balittanah [27]*

Conclusions

The third mature leaves at harvest of non-fruiting branches can be used to diagnose of N, P and K status in lansium tree. N, P and K concentration has the best correlation with fruit yield, while the third or first mature leaves at harvest time of fruiting branches can be an alternative to diagnose N, P and K status of duku plants when all branches produced fruit.

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Exploitation of Variability and Genetic Divergence of Tomatillo (*Physalis ixocarpa* Brot.) as Tool for Further Breeding

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Abstract

Physalis ixocarpa Brot. has potential to become an important fruit in Indonesia. It contains nutritional good value for health and reduces malnutrition problem. The aims of this study were to evaluate the performance of set genotypes of tomatillo for yield and fruit quality and to identify variability for important characteristics. The experiment was conducted in Areng-Areng, Junrejo, Batu City East Java. Thirty-two genotypes of tomatillo (*Physalis ixocarpa* Brot.) were included to study the genetic divergence. Twenty-three characteristics were used to describe variability within the genotypes. Clustering of genotypes was done through agglomerative hierarchical clustering based on agglomeration method unweighted pair-group average and the similarity Pearson correlation coefficient. The first five principal components were 48.73%, 19.52%, 6.44%, 6.13% and 4.58% and cover 85.40% of the total variance. Plant characteristics which contributed to total variation in tomatillo were height at first branch, number of tertiary branches, number of flowers per branch, number of fruits per plant, fruit weight with calyx per plant, calyx length, calyx diameter, fruit diameter, average of fruit weight with calyx, average of fruit weight without calyx, fruit weight without calyx per plant, dentation of margin, leaf blade colour, fruit depth of stalk cavity, fruit main colour (at harvest maturity), fruit intensity of main colour (at harvest maturity), fruit main colour (at physiological maturity), and fruit flesh colour. Clustering grouped the plant 32 genotypes into six clusters.

Keywords: Physalis ixocarpa*, tomatillo, genetic divergence, variability*

Introduction

Physalis ixocarpa Brot., is a member of the Solanaceae family, commonly known as tomatillo. It is an annual herb crop; tomatillo has variations on morphological characters that can be used to differentiate varieties [1]. The fruit is similar to tomato but it is covered by husk, and has a green, yellow-green [2, 3], violet or purple [4, 5] colour. This plant has wide range of adaptation, from the humid tropical, subtropical condition, and temperate region [6, 7]. Fruits are covered by the "husk" (the calyx) and are also known as husk tomato. Tomatillo is cultivated for fruits consumption.

Although, their roots, stems, leaves, and calyxes have important variety of antioxidant that can be utilized [8]. The fruits have a good nutritional value, they contain vitamin A and in higher amounts vitamin C, B, B_2, carotenoids, iron, and polyphenols [4, 9]. Tomatillo contains choline and flavonoid compounds belonging to vitamin P [10]. It also has been reported that chemicals present in tomatillos, e.g., ixocarpalactone A, may have cancer chemo preventive properties [11].

Most tomatillos are slightly sour and sweet at the same time. The domination of citric acid on fresh tomatillos makes it taste sour [12]. In Mexico and Central America, the fruits are widely used in popular cuisine like salads, soups, stews and green sauces [13]. In Poland, it is processed into a jam, relish, with zucchini and hot pepper [9]. Utilization of the nutritional potential of tomatillo fruit can help to reduce malnutrition problems and enhancing the availability of fruit and vegetables in Indonesia. Recently, the demand for the fruit of *Physalis* has increased due to the high market opportunity, a small number of producer and exuberant market price [14]. People awareness about healthy food contributes to the high demand of *Physalis* fruit that is rich in health-promoting substances and antioxidant. Recent scientific evidence pointed out that tomatillo contains a high level of antioxidants including vitamin C and phenolic compounds [4].

Tomatillo is newly introduced as a commercial crop in Indonesia because of its excellent market possibilities, its versatility for processing and its adaptability to different growing conditions. Different *Physalis* species are produced in Indonesia (e.g., *Physalis angulata* L., *Physalis minima* L., and *Physalis peruviana* L) but *P. ixocarpa* Brot. has the biggest potential to become an important fruit crop. In respect to the potential use of *P. ixocarpa* the study of the morphological, chemical and genetic diversity of this species is of great importance for further improve quality and yield of this *Physalis* species. The tomatillo diversity in Indonesia can occur from the transfer of plant genetic material through trade or exchange between institutions or countries. Hybridization between genotypes will expand tomatillo characters variability [15].

Genetic diversity and morphological variability in *P. ixocarpa* have not previously been evaluated in Indonesia. Breeding tomatillo plants in Indonesia can be done by managing characters genetic variability. Measurement of genetic variability can be initiated by genotypic separation followed by identification of the character of each genotype. Genotypes which identified according to consumer tastes can be channelled to consumers or increased genetic capacity through plant breeding techniques and technology. Plant breeding technology that can be used in tomatillo is hybridization [15], mutation [16], polyploidy [6, 17-20], and genetic engineering [21].

In this study, we, therefore, evaluated the performance of set genotypes of tomatillo for yield and fruit quality and identification of the character variability.

Materials and Methods

The field experiment was conducted in Areng-Areng, Junrejo, Batu City East Java during the period May to September 2017. Thirty-two genotypes of tomatillo (*Physalis ixocarpa* Brot.) originated from Malang and Tangerang were evaluated. Data recorded from five plants per genotype including height at first branch, number of tertiary branches, number of flowers per branch, number of flowers per plant, number of fruits per plant, fruit weight with calyx per plant, calyx length, calyx diameter, fruit diameter, fruit length, average of fruit weight with calyx per plant, average of fruit weight without calyx per plant, fruit total soluble solid, fruit acidity, fruit weight without calyx per plant, intensity of anthocyanin coloration of internodes, dentation of margin, leaf blade colour, fruit depth of stalk cavity, fruit main colour (at harvest maturity), fruit intensity of main colour (at harvest maturity), fruit main colour (at physiological maturity), and fruit flesh colour [22]. The average fruit weight was measured per fruit. The criteria of coefficient of variation (CV) was high (more than 25%), while moderate (10-25%) and low (<10%) [23].

Principal component analysis (PCA) was used to study the variability within the genotypes and to cluster the genotypes. The dendrogram was generated through agglomerative hierarchical clustering based on agglomeration method unweighted pair-group average and the similarity Pearson correlation coefficient.

Results and Discussions

The study revealed that different genotypes of *Physalis ixocarpa* Brot. exhibited a large variability for the morphological studied (Table 1). The highest standard deviation was observed for fruit weight without calyx per plant (g) followed by fruit weight with calyx per plant, average of fruit weight with calyx, average of fruit weight without calyx, number of flowers per plant, and number of tertiary branches. The lowest standard deviation was noted for fruit total soluble solid.

The variation among genotypes for evaluated characteristics expressed as percent coefficient of variation. The largest variation was observed for fruit depth of stalk cavity with CV of 58.47% followed by leaf blade colour (57.19%), fruit length (52.97%), fruit main colour (at physiological maturity) (47.76%), number of tertiary branches (43.97%), average of fruit weight with calyx (39.59%), average of fruit weight without calyx (38.75%), fruit main colour (at harvest maturity) (38.11%), fruit weight without calyx per plant (38.08%), and fruit weight with calyx per plant (37.70%). Variation in colour among tomatillo genotypes has already been reported by [24, 25].

The main fruit colour of tomatillo genotypes varies between green and purple (Fig. 1).

Tomatillo berries can be varied between 1-10 cm in diameter [26], result based on our genotype collection showed that fruit diameter of tomatillo genotypes range from 2.94-4.88 cm (Fig. 1).

Fruit total soluble solid has shown the least variation with CV of 2.83%. Yield and fruit quality analysis based on different plant characteristics showed a high genetic divergence between the 32 tomatillo genotypes, which could be used for future quantitative and qualitative breeding programs and as a source for health foods.

Table 1. Character variation of 32 genotypes of tomatillo

Characters	Min	Max	Mean	Std. dev	CV (%)
Height at first branch (cm)	10.00	18.00	13.76	2.36	17.12
Number of tertiary branches	12.00	48.00	26.00	11.43	43.97
Number of flowers per branch	6.60	18.98	12.46	3.39	27.22
Number of flowers per plant	49.00	87.00	67.84	11.68	17.22
Number of fruits per plant	33.00	49.00	41.41	4.43	10.69
Calyx length (cm)	3.23	4.43	3.69	0.35	9.36
Calyx diameter (cm)	3.07	4.98	4.12	0.66	16.11
Fruit diameter (cm)	2.94	4.88	3.96	0.64	16.08
Fruit length (cm)	2.68	10.15	4.11	2.18	52.97
Average of fruit weight with calyx (g)	13.95	48.62	32.60	12.91	39.59
Average of fruit weight without calyx (g)	13.56	44.09	31.43	12.18	38.75
Fruit total soluble solid (Brix)	10.61	12.22	11.63	0.33	2.83
Fruit acidity (pH)	2.66	4.28	3.56	0.51	14.40
Fruit weight with calyx per plant (g)	455.12	1794.00	1029.43	388.12	37.70
Fruit weight without calyx per plant (g)	392.38	1731.02	998.96	380.41	38.08
Intensity of anthocyanin coloration of internodes	3.00	5.00	3.50	0.88	25.14
Dentation of margin	2.00	3.00	2.63	0.49	18.74
Leaf blade colour	1.00	3.00	1.69	0.97	57.19
Fruit depth of stalk cavity	1.00	3.00	1.56	0.91	58.47
Fruit main colour (at harvest maturity)	2.00	5.00	3.28	1.25	38.11
Fruit intensity of main colour (at harvest maturity)	1.00	2.00	1.72	0.46	26.58
Fruit main colour (at physiological maturity)	2.00	5.00	3.03	1.45	47.76
Fruit flesh colour	3.00	5.00	3.69	0.97	26.17

Std. dev standard deviation, *CV* coefficient of variation

The PCA reveals the importance of the largest contributor to the total variation at each axis of differentiation [27]. The first five principal components (PC) having greater than one eigenvalue contributed 85.40% to the total variation among 32 genotypes of tomatillo (Table 2). PC1 contributed for about 48.73%, whereas PC2, PC3 PC4, while PC5 contributed 19.52%, 6.44%, 6.13%, and 4.58%, respectively to the total variation.

The characteristics, which contributed to total variation at each PC has factor loading > |0.6| [28].

The characteristics which contributed to PC1 were height at first branch, fruit weight with calyx per plant, calyx length, calyx diameter, fruit diameter, average of fruit weight with calyx, average of fruit weight without calyx, fruit weight without calyx per plant, leaf blade colour, fruit depth of stalk cavity, fruit main colour (at harvest maturity), fruit intensity of main colour (at harvest maturity), fruit main colour (at physiological maturity), and fruit flesh colour. Number of tertiary branches, number of flowers per branch, dentation of margin, fruit depth of stalk cavity, and fruit intensity of the main colour (at harvest maturity) had shown more contribution to PC2. Number of fruits per plant has contributed to PC4. Thus, these results indicated that the plant characteristics referred above are suitable breeding purposes.

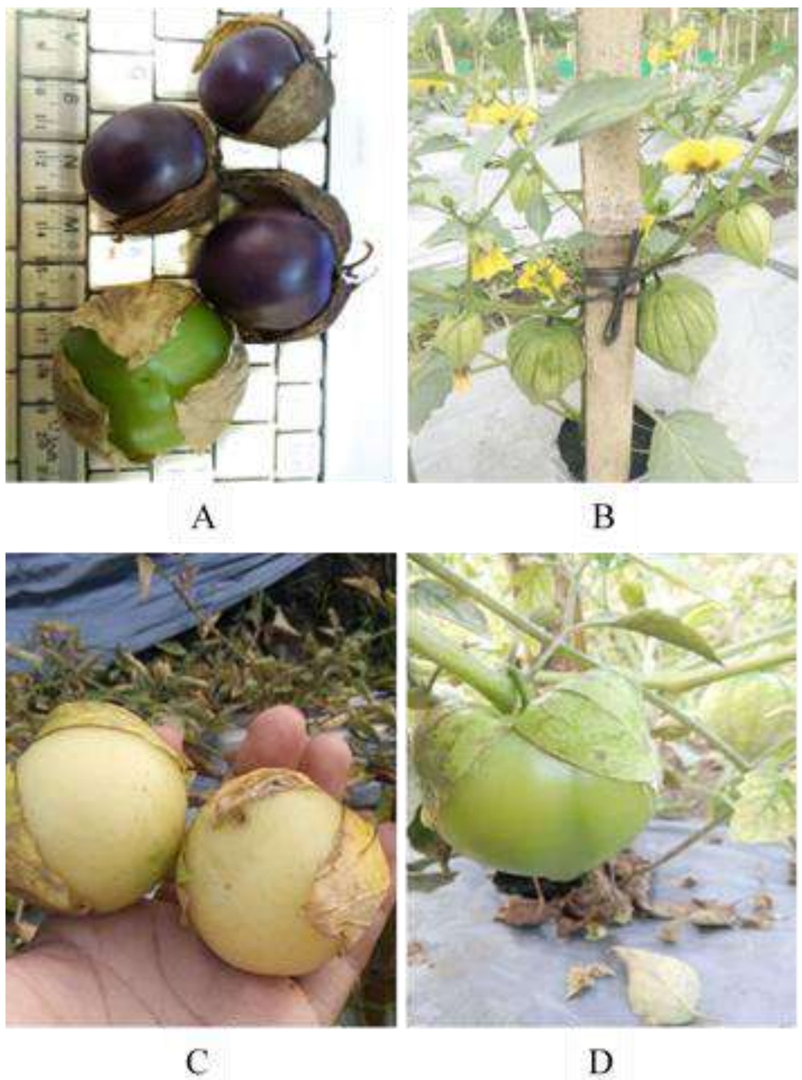

Fig. 1. Fruit main colour of tomatillo genotypes and the differences of tomatillo fruit size (A-D).

Table 2. Principal component analysis for characters of 32 tomatillo genotypes

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	11.21	4.49	1.48	1.41	1.05
Variability (%)	48.73	19.52	6.44	6.13	4.58
Cumulative (%)	48.73	68.25	74.69	80.82	85.40
Characters					
Height at first branch (cm)	**-0.72**	0.14	0.12	0.24	-0.01
Number of tertiary branches	-0.46	**0.84**	-0.01	-0.13	-0.02
Number of flowers per branch	-0.48	**0.79**	0.00	0.01	-0.18
Number of flowers per plant	-0.30	-0.11	0.08	0.54	0.57
Number of fruits per plant	0.04	-0.28	0.14	**0.71**	-0.33
Calyx length (cm)	**-0.83**	0.25	-0.16	-0.16	0.27
Calyx diameter (cm)	**-0.90**	-0.37	-0.18	0.02	-0.01
Fruit diameter (cm)	**-0.90**	-0.36	-0.20	0.01	0.01
Fruit length (cm)	-0.59	0.48	0.03	0.14	-0.33
Average of fruit weight with calyx (g)	**-0.91**	-0.35	-0.13	-0.09	-0.01
Average of fruit weight without calyx (g)	**-0.93**	-0.29	-0.10	-0.09	-0.03
Fruit total soluble solid (Brix)	-0.09	-0.47	0.53	-0.34	-0.01
Fruit acidity (pH)	0.02	0.22	-0.52	0.39	-0.35
Fruit weight with calyx per plant (g)	**-0.74**	-0.03	0.58	0.17	-0.07
Fruit weight without calyx per plant (g)	**-0.73**	0.03	0.59	0.17	-0.08
Characters					
Intensity of anthocyanin coloration of internodes	0.44	-0.10	0.16	-0.33	-0.48
Dentation of margin	0.29	**0.91**	0.12	0.01	0.12
Leaf blade colour	**0.95**	0.28	0.09	0.07	0.06
Fruit depth of stalk cavity	**-0.68**	**0.68**	0.04	-0.07	0.07
Fruit main colour (at harvest maturity)	**0.90**	-0.05	0.07	0.12	0.10
Fruit intensity of main colour (at harvest maturity)	**0.68**	**-0.68**	-0.04	0.07	-0.07
Fruit main colour (at physiological maturity)	**0.95**	0.28	0.09	0.07	0.06
Fruit flesh colour	**0.95**	0.28	0.09	0.07	0.06

Clustering of genotypes based on studied characteristics studied is presented in Fig. 2. Cluster analysis grouped the 32 genotypes into six clusters as shown in Table 3. Cluster I comprised of 11 genotypes viz. P14, P15, P35, P13, P22, P23, P32, P33, P34, P12, and P21. Cluster II comprised of 12 genotypes followed by 6 genotypes in Cluster III. Cluster IV, V, and VI had only one genotype respectively. The first cluster was characterized by a minimum height at first branch, calyx length, calyx diameter, fruit diameter, fruit length, average of fruit weight with calyx, and average of fruit weight without calyx (Table 3). Therefore, the genotypes in cluster I had the lowest average values for fruit quality. The second cluster was characterized by maximum yield and fruit quality in characteristics of height at first branch, number of tertiary branches, number of flowers per branch, fruit weight with calyx per plant, calyx length, fruit length, and fruit weight without calyx per plant. Genotypes in the same cluster showed relationship closeness based on yield and fruit quality characters. The genotypes from the same cluster can be used for improving desired characters through hybridization to obtain high yield and good fruit quality varieties. The genotypes from different cluster could be utilized in tomatillo improvement programs for introducing desired yield and fruit quality characters.

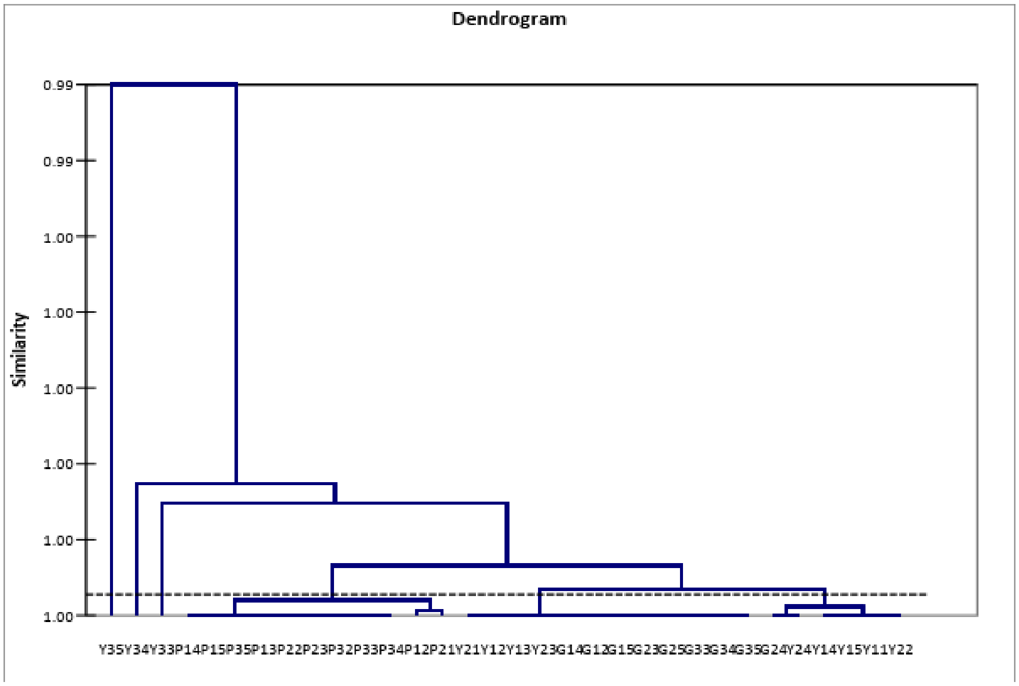

Fig. 2. Dendrogram of 32 tomatillo genotypes based on yield and fruit quality characters

Table 3. Mean values or mode of different characters of tomatillo genotypes based on cluster analysis

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Height at first branch (cm)	11.76	15.63	13.92	13.00	14.00	13.00
Number of tertiary branches	22.73	33.50	22.00	17.00	15.00	16.00
Number of flowers per branch	11.37	14.59	11.31	11.35	10.53	8.80
Number of flowers per plant	64.18	70.25	66.50	82.00	78.00	63.00
Number of fruits per plant	41.45	40.92	40.83	47.00	44.00	42.00
Calyx length (cm)	3.35	3.93	3.79	3.77	3.78	3.87
Calyx diameter (cm)	3.24	4.50	4.59	4.98	4.80	4.87
Fruit diameter (cm)	3.13	4.32	4.37	4.88	4.72	4.72
Fruit length (cm)	2.90	5.63	3.60	3.51	3.49	3.54
Average of fruit weight with calyx (g)	15.64	39.93	42.10	44.92	48.62	45.94
Average of fruit weight without calyx (g)	15.35	39.22	40.30	43.63	42.69	38.17
Fruit total soluble solid (°Brix)	11.55	11.67	11.81	11.23	11.30	11.61
Fruit acidity (pH)	3.60	3.51	3.57	4.12	3.18	3.56
Fruit weight with calyx per plant (g)	684.70	1457.25	896.95	654.00	1415.00	472.31
Fruit weight without calyx per plant (g)	671.81	1429.87	858.10	635.22	1242.23	392.38
Intensity of anthocyanin coloration of internodes	weak	weak	weak	weak	weak	weak
Dentation of margin leaf blade	strong	strong	medium	medium	medium	medium
Leaf blade colour	purplish green	yellowish green	yellowish green	yellowish green	yellowish green	yellowish green
Fruit depth of stalk cavity	absent	shallow	absent	absent	absent	absent
Fruit main colour (at harvest maturity)	purple	green	yellow	yellow	yellow	yellow
Fruit intensity of main colour (at harvest maturity)	intermediate	light	intermediate	intermediate	intermediate	intermediate
Fruit main colour (at physiological maturity)	purple	green	green	green	green	green
Fruit flesh colour	purplish green	greenish yellow	greenish yellow	greenish yellow	greenish yellow	greenish yellow

Conclusion

It can be concluded that the variability in height at first branch, number of tertiary branches, number of flowers per branch, number of fruits per plant, fruit weight with calyx per plant, calyx length, calyx diameter, fruit diameter, average of fruit weight with calyx, average of fruit weight without calyx, fruit weight without calyx per plant, leaf blade dentation of margin, leaf blade colour, fruit depth of stalk cavity, fruit main colour (at harvest maturity), fruit intensity of main colour (at harvest maturity), fruit main colour (at physiological maturity), and fruit flesh colour were significant. Genotype clustering techniques grouped 32 genotypes into six clusters in relation to important characteristics which are linked to yield and quality. The study shows that genetic divergence can be used to improve genotypes.

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Macronutrient Analysis and Secondary Metabolit Compounds of Tin Fruits (*Fiscus* Carica Linn) from Poso, Central Sulawesi

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Abstract

The figs come from Middle Eastern countries namely Mecca, Palestine, Egypt and Turkey.

These plants can grow in sub-tropical climates, heat to dry. In Poso, Central Sulawesi, fig fruit plants thrive and are widely cultivated. The purpose of this study was to analyse macronutrient levels and identification of secondary metabolites in figs found in Poso, Central Sulawesi.

Macronutrient analysis in this study was carried out quantitatively on carbohydrate content using Lane Eynon, fat by Babcock method and protein by spectrophotometer. While the analysis of secondary metabolites carried out qualitatively includes: flavonoids, alkaloids, steroids and terpenoids. The results of quantitative carbohydrate analysis were 20.25g, 0.75g and 0.28g in 100g sample weight, respectively. The results of analysis secondary metabolites detected flavonoids, alkaloids, steroids and terpenoids.

Keywords: Fruit Tin, macronutrient compounds, secondary metabolite

Introduction

Indonesia is an enchanting country with a tropical climate, so that diverse plants can thrive.

The fig which is a commodity that is in demand by many people from Middle Eastern countries namely Makkah, Palestine, Egypt, and Turkey can also grow in tropical regions [1].

Around 2004, Green Jordan and Purple Jordan figs entered Indonesia, then in June 2006 Negronne, Black Ischia, Long Yellow, Genoa White, Conadria, Calymirna, Red Libya, and Red Palestine. In May 2010 the types of hybrids from France were Bourjassote, Noire de Caromb, Madeline, Dhoupine, Tena, Abicue, and barbera, the type of fig also entered Indonesia [2].

The fig has long been known to have its benefits as an herbal medicine because its compounds are believed to cure various diseases [3]. In general, part of the fig has been widely used as an antioxidant and anticancer. tin also contains bioactive components such as phenol, benzaldehyde and flavonoids. Flavonoid compounds are polyphenol groups commonly found in plants and are pigments in higher plants [4]. This compound is found in all parts of the plant, including fruit, pollen and roots [5]. According to Miller [6], a number of medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-allergic and anti-allergic activities [7].

This fig plant in Central Sulawesi Province has been cultivated in Bombana, Buton, North Buton, Kolaka, North Kolaka, Konawe, South Konawe, North Konawe, Muna, Wakatobi, Bau-Bau, Kendari, Poso, Banggai, Buol, Morowali and TojoUna-Una, but not yet widely known by the people of Central Sulawesi what is the benefit of this fruit, so that by the community only as an ornamental plant. A very interesting problem is whether the fig plants that grow in the center of Central Sulawesi have fruits that have the same macro nutrient content and secondary metabolites as figs from the Middle East, and can be used as traditional medicine, so it becomes very important for informed to the public, especially Central Sulawesi. This aims of study to describe the results of macro nutrient analysis and secondary metabolite compounds in figs originating from Poso, Central Sulawesi

Methodology

1. Tools and materials

The tools used in this study: digital balance (Kern kb), 100 mL measuring cup, 1000 mL measuring cup, 250 mL beaker, 100 mL beaker, 50 mL measuring flask, 100 mL measuring flask, test tube, test tube rack , separating funnel, stirring rod (Gemmy), electric bath (Barnstead), Erlenmeyer 200 ml, 500 ml Erlenmeyer, orbital shaker (Gerhandti), magnetic stirrer (Eyela), blender, aluminium foil, tissue, scissors, Babcock bottle, spectrophotometer array Miltonroy 3000).

The ingredients used 70% ethanol (E.Merck), distilled water, hexane (Technical), label paper, filter paper, figs, concentrated HCl solution (E.Merck), Mg metal, concentrated H_2SO_4 solution (E. Merck), Pb. Acetate, Na. Oxalate, Soxhlet reagent, amido Black, Lowry reagent, Anthrone reagent and Mayer reagent (making reagents according to Sudarmadji, *et al*., [8].

2. Research procedure (Fat, Protein and Carbohydrate)

Ripe and fresh Tin were prepared, then washed thoroughly, peeled, then cut into small pieces, left for a while until wilted (he moisture content decreases).

Macronutrient tests included fats, proteins and carbohydrates.

2.1 Fats

The blended fruit is weighed as much as 20 g, then put it in a Babcock bottle and add 20 mL of H_2SO_4, stirring until smooth and perfectly mixed. Babcock bottles were installed in a centrifuge and matched the Babcock bottle the same weight, and rotated for 5 minutes. After that water was added until the pumpkin from the Babcock bottle was fully filled, then centrifuged for 2 minutes.

More hot water was added until the liquid fat is on the neck of the bottle that is aligned and centrifuged again for 1 minute. The bottle was put in hot water for 10 minutes and tried to make the fat surface the same as the hot water surface. Bottles containing fat were dried, then the fat is measured [8].

2.2 Protein

A standard solution using a BSA protein (Bovine Serum Albumin) as much as 300 µg/mL was prepared. The solution was then put into a reaction tube with levels of 30-300 µg/mL, carried out in Sudarmaji *et al*., [8]. Then a 1 mL sample was measured, put into a centrifuge tube, and centrifuged for 10 minutes at a speed of 11,000 rpm (content measurement protein is carried out as in the Sudarmaji procedure [8], [10], [11].

2.3 Carbohydrate

Determination of carbohydrate content was done by adding 1 g of sample with 10 mL of distilled water then filtered using filter paper. Next the filtrate was diluted to 100 mL, taken as much as 2 mL and then added with 4 mL of Anthrone reagent. The solution was heated for 10 minutes, then the solution was cooled in a cup containing water and absorbance was measured at a wavelength of 630 nm using a UV-Vis spectrophotometer [8], [12].

Subsequent treatments were made a standard solution of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm by means of 0.1 g of glucose solids and put it into a 100 mL volumetric flask and added distilled water to the boundary markings. For 10 ppm taken as much as 0.5 mL, for 20 ppm taken as much as 1 mL for 30 ppm taken as much as 1.5 mL, for 40 ppm taken as much as 2.0 mL and for 50 ppm taken as much as 2.5 mL and each was put into a 50 mL volumetric flask. Then added aqudes to the boundary mark. The standard solution was given the same treatment as the sample solution to analyse the glucose content.

3. Secondary Metabolite Test

3.1 Make extracts of figs with 3 kinds of solvents

A total of 30 grams of fig were weighed and put into three Erlenmeyer (I-III). After that 100 mL of distilled water was added to Erlenmeyer I, 100 mL of 70% ethanol in Erlenmeyer II, and 100 mL of hexane in Erlenmeyer III. Then the three Erlenmeyer’s were covered with aluminium foil, left to submerged for 2 hours, then dished for 4 hours. After that, all three extracts are filtered.

The obtained filtrate was used for the secondary metabolite test sample.

3.2 Secondary Metabolite Test of Tin Fruit Extract

Testing of alkaloid and flavonoid compounds followed to the work procedures of Nuryanti, and Puspitasari [13], [14], [15], [16]. The testing stages ware as follows:

3.2.1 Alkaloid Test

The extract of each fig was taken as much as 2 mL, then put into 3 different test tubes. After that each extract was added 3 drops of concentrated hydrochloric acid, and 5 drops of Mayer reagent. If each solution was formed white precipitate then the positive sample contains alkaloids [13], [14].

3.2.2 Anthocyanin

Each of the fig extracts was taken a few drops and placed on 3 plates of thin layer chromatography (TLC), then evaporated with NH_3 vapor, blue discoloration would occur if the extract contained anthocyanin [15].

3.2.3 Test for flavonoids

Extracts of each fig were taken as much as 2 mL and put into 3 test tubes, then heated for about 5 minutes. After heating, each of them was added with 0.1 gram of Mg metal and 5 drops of concentrated HCl. If each solution is orange to red, it is positive for flavonoids [13].

3.2.4 Terpenoid Test

Extracts of figs each were taken as much as 2 and put into 3 test tubes. After that, each extract was added with 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4. If each solution in the tube is formed red or purple then it is positive to contain terpenoids [13].

3.2.5 Steroid Test

Extracts of figs were taken as much as 2 mL each, put into 3 test tubes, after which each extract was added with 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4. If each solution is formed in green, it is positive for steroids [13], [16].

Results and Discussion

1. Macronutrient Tin fruit

The results showed that the macro nutrient content in figs in Poso, Central Sulawesi were as follows: carbohydrates 20.25g, fat 0.75g and protein 0.28g in 100 g samples. When compared with figs from Turkey almost the same (karbohirat 19.18 g, total fat 0.30 g, protein 0.75 g., Crisosto *et al*., [17]. Research samples can be categorized as having similar nutrient content as Green Jordan, which is from Middle Eastern countries, because it has the same physical properties and macro content of nutrients as stated by Crisosto *et al*., [17].

Figs have different characteristics Green Jordan fruit flesh is slightly white and in the middle is red and has many small seeds. Jordan PurpPurpel fruit is slightly yellow and pale red and has many small seeds. Tin Negronne flesh is reddish purple and has small seeds in the middle. Tin brown Turkey the colour of the flesh is fresh red [17]. From the results of the study, if we look at the levels of macro nutrients in figs in Poso, Central Sulawesi, the quality is not much different from figs originating from Middle Eastern countries.

2. Fruit Tin Secondary Metabolite Test

The results of the testing of secondary metabolites on extracts of fig using several solvents namely distilled water, 70% ethanol and hexane can be seen in Table 1.

Table 1. Secondary Metabolite Test Results of Figs Extract

No	Extract of Figs in solvent	Result of experiment				
		Alkaloid	Anthocyanin	Flavonoid	Terpenoid	Steroid
1.	aquadest	+	+	+	-	-
2.	Ethanol 70%	+	+	+	-	-
3.	Hexana	-	+	-	-	+

Keterangan: (+) = ada, (-) = tidak ada

Alkaloid testing is carried out using Mayer reagents, giving positive results which are characterized by the formation of white deposits. Based on Table 1, it was found that extracts of fig with solvents of distilled water and 70% ethanol were positively containing alkaloids, whereas for hexane did not contain alkaloids. The positive results of alkaloids in the Mayer test are characterized by the formation of white deposits. It is estimated that the precipitate is a potassium-alkaloid complex. Alkaloid test with Mayer reagent, it is estimated that nitrogen in the alkaloid will react with K^+ ion from K-tetraiodomerkurat (II) potassium to form a precipitating potassium-alkaloid complex. Estimates of the reactions that occur in the Mayer test are shown in Figure 1 below:

$$HgCl_2 + 2KI\,HgI_2 \longrightarrow +2KCl$$
$$HgI_2 + 2KI \longrightarrow K_2[HgI_4]$$

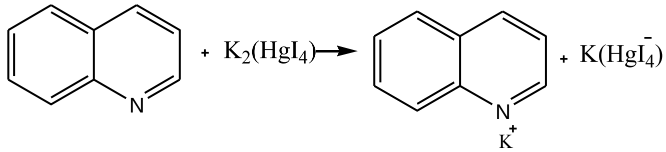


Fig. 1. Estimated Mayer Test Reaction [13], [14]

The anthocyanin test results showed that extracts with alcohol solvents and water contained anthocyanins, because they were blue. Anthocyanin, a natural compound that is slightly acidic, so that the base will react like an acid base reaction. The reaction that occurs is shown in Figure 2.

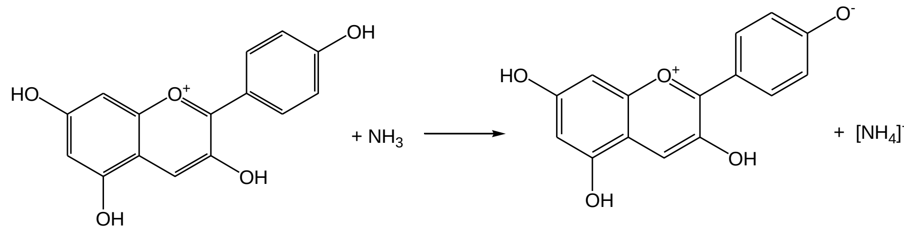
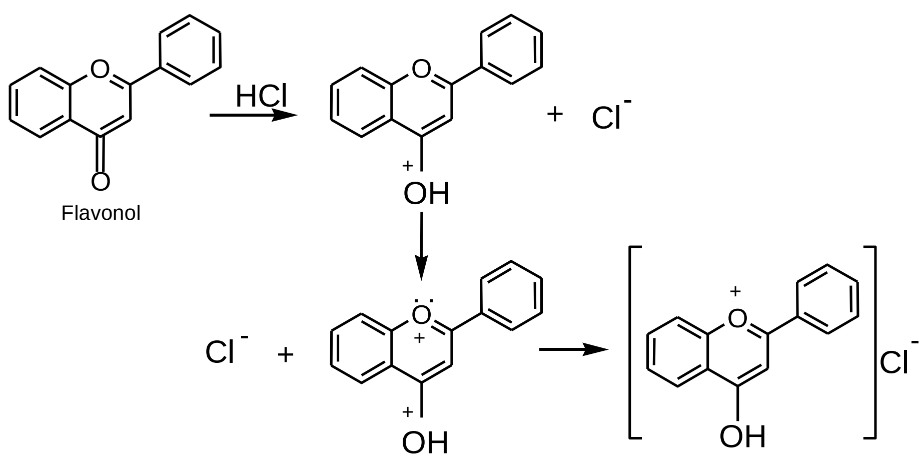


Fig. 2. Reaction of Anthocyanin Test [14], [15]

The results of flavonoid testing from extracts of figs with solvents of distilled water and 70% ethanol were the formation of a yellow solution which indicated the presence of flavonoid compounds. The function of heating is to dissolve flavonoid compounds, because flavonoids can dissolve in hot water. The addition of Mg and HCl metal is to reduce the benzopyrone nucleus contained in the flavonoid structure to form red or orange flavylium salt. Flavonoids according to their structure are derived from flavone parent compounds. Flavonoids are compounds containing two aromatic rings with more than one hydroxyl group. flavonoid compounds that are soluble in water, so that flavonoids can be extracted with 70% ethanol (ethanol containing about 30% water).

The flavylium salt formation reaction is shown in Figure 3.



red

Fig. 3. Reaction of Flavilium Salt Formation [13]

Flavonoids are found in all parts of the plant including leaves, wood roots, bark, pollen skin, nectar, flowers, fruit, and seeds [18]. Only a few records report the presence of flavonoids in animals, for example in the smell glands of beavers, propolis (bee secretions) and inside the wings of butterflies, and even then, with the assumption that these flavonoids come from plants that feed the animals and are not biosynthesized in animal body [19], [20].

Terpenoid and steroid testing using Liebermann-Burchad reagent, i.e., a mixture of concentrated HCl with concentrated H_2SO_4. This analysis is based on the ability of terpenoids and steroids to form colours by concentrated H_2SO_4 in a hydrochloric acid solvent. Positive results are shown in the form of orange red for triterpenoid and green analysis for steroid analysis [18], [23], [20].

The results of the testing of terpenoids and steroid compounds in each extract of figs with solvents of distilled water and 70% ethanol contained no terpenoids and steroids. As for the extract of figs with hexane solvents there are steroid compounds. Hexane is a nonpolar solvent and terpenoid and steroid compounds are non-polar compounds so that these compounds can be extracted perfectly in the solvent [24], [25].

Conclusion

Figs from Poso, Central Sulawesi have carbohydrate content of 20.15g, fat of 0.75g and protein of 0.28g. The content of the macronutrients is almost the same as the figs from Middle Eastern countries. Figs are also identified as containing secondary metabolites such as flavonoids, anthocyanins, alkaloids, terpenoids and steroids.

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"INA 03", Promising Banana Cultivar for Fusarium Wilt Control

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Abstract

Panama disease is one of important banana diseases which is caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*). This disease is very difficult to be controlled due to its spread that can be occurred easily through soil, water, planting material and human activities. Various methods of diseases controls have been applied both technical practices and biological control. However, biological control in the laboratory scale that has been proven effective, not necessarily effective in the field. In addition, it must also be economical in its application. The use of resistant cultivar is an alternative to overcome the fusarium wilt. In order to produce fusarium resistant cultivar, Indonesia Tropical Fruit Research Institute (ITFRI) has carried out banana conventional breeding since 2000. Currently, one banana hybrid has been obtained and proven to be resistant to fusarium wilt on the field, namely INA 03. In addition, this hybrid INA 03 also has superior characteristics, such as semi-dwarf stature with a height between 2-2.5 m, and high TSS reaching 29 Brix. After going through several stages of evaluation INA 03 has been registered as a new superior cultivar, so it can be distributed in the farmers.

Keywords: Banana, conventional breeding, Fusarium wilt, resistant cultivar

Introduction

Banana is one of fruit commodities that are consumed by people around the world. Bananas are mostly produced by developing countries in Asia, Africa and Latin America, with a total production reaching 148.43 million tons in 2016 (https://bit.ly/2zNFBYg). Of the 10 largest banana-producing countries, India ranked first with an average production of 27.5 million tons in 2016, followed by China, Uganda, the Philippines, Brazil, Ecuador, Indonesia, Colombia, Cameroon and Ghana.

Bananas play a role in food security programs, especially in African countries such as Uganda, Rwanda and Cameroon. Banana is one of the staple foods in those countries with consumption levels more than 200 kg/capita/year. Meanwhile banana consumption of Indonesian people is still very low at 6.02 kg/capita/year of fresh fruit. The Indonesian people consider banana to be a fruit not as one of staple foods, which will be eaten if deemed necessary. However, the consumption of banana is still higher than consumption of other fruits. The result of national food survey showed that in 2015 total fruit consumption per year was 6.02 kg for dessert bananas, while oranges were 3.28 kg and papaya 2.25 kg per capita/year (http://gizi.depkes.go.id/wp-content/uploads/2017/01/Paparan-BPS-Konsumsi-Buah-Dan-Sayur.pdf).

This paper will explain the process of obtaining INA 03. A new banana cultivar that resistant to Fusarium wilt, started from parent's selection, flower pollination, embryo rescue, field evaluation until the registration of selected progeny as a superior new cultivar, as well as further steps will be taken after being released as new cultivars.

Panama Disease in Indonesia

The soil-borne fungus Fusarium oxysporum f. sp. cubense (Foc) causes Fusarium wilt, one of the most important lethal diseases in banana [1] Ploetz, R. C., 2015; [2] Dita *et al*., 2018) which has a devastating effect on banana production. Fusarium wilt has become a worldwide concern because it has wiped out banana plantations in several banana producing countries, including in Indonesia. The massive destruction of commercial banana plantations by fusarium wilt (*Foc*) was occurred in the 1950s in Latin America [3] Stover & Simmond, 1987). The banana cultivar destroyed was Gros Michel and the pathogen that were identified as *Foc* race 1. The pathogen also attacked several banana cultivars in the Asian region such as pisang Awak and Rasthali or Raja Sere. Other race of *Foc*, race 2 attacks cooking bananas such as Bluggoe and related plantain. *Foc* race 4 is the most virulent pathogen which in the tropics attacked the susceptible and resistant cultivars to race 1 and race 2. *Foc* tropical race 4 has destroyed Cavendish plantations in China, Taiwan, the Philippines, Australia and several cultivars in Indonesia and Malaysia.

Based on the compatibility group, *Foc* is classified into VCGs (vegetative compatibility groups). At present 24 VCGs have been identified and 21 of them were found in Australia and Asia [4] (Ploetz & Correll 1988; [5] Mostert *et al*., 2016). Seven VCGs were rediscovered in Indonesia through the surveys conducted by ITFRI in 2006-2007 on Sumatra, Java, Kalimantan, Sulawesi, Maluku and Papua [6] Riska & Hermanto, 2012). Of the seven VCGs, VCG 01213/16 isolates were found in almost all islands in Indonesia. VCG 01213/16 isolate is the member of *Foc* tropical race 4 (TR4). Most of Indonesian commercial banana cultivars were susceptible to *Foc* TR4, such as Barangan, Ambon Hijau, Ambon Kuning, Raja Sere, Mas and others. Xiao *et al*., (2013) [7] said *Foc* 4 progresses in the vascular tissue of banana roots to the pseudostem, which correlates with *Foc* pathogenicity, is not clearly known. One of the methods to control *Foc* is using disease resistant banana cultivars. *Foc* resistant can be obtained by selection of existing banana germplasm or by cross pollination among banana cultivars or accessions [8] (Rowe & Richardson, 1975).

Banana Breeding in ITFRI

Banana breeding program can be carried out conventionally or unconventionally such as genetic transformation or mutation breeding [9] (Okole *et al*., 2000). Even though the conventional breeding is difficult due to the problem of compatibility and fertility of banana flowers, some countries such as Honduras, Brazil and India have produce disease-resistant cultivars [10] Moran, 2013; [11] Menon *et al*., 2011; [12] de Oliveira e Silva, 2001; [13] Poerba *et al*., 2017; [14] Oselebe *et al*., 2010; [15] Crouch *et al*., 1999. One of hybrids was obtained from conventional breeding that have been widely spread around the world is Gold Finger or FHIA-01 [16] (Rowe, 1990).

Selection of Parent Plants

Banana breeding programs in order to overcome fusarium wilt were started in 2000. The method used was conventional plant breeding through hybridization between banana accessions or cultivars. As a first step, the selection of parents consisting of commercial cultivars and wild species that were proved to be resistant to panama wilt. All the banana accessions used in this study were Barif 0130 (AAw), Barif 0004 (AAw), Barif 0013 (AAw) and Barif-0012 (BB) [17] (Edison *et al*., 2004). As female parents were commercial cultivars that will be improved, namely Barif 0028 (AAA), Barif-0014 (AAA), Barif 0024 (AAA), Barif 0025 (AA), Barif 0029 (AAA), Barif 0033 (AAB).

Barif 0130 is an introduced accession from the International Transit Center (Bioversity International) in 1995 labeled as ITC 0249, which is a wild banana species native to India. This accession showed resistant to panama disease under field evaluation. The advantages of this accession are having a large number of fertile pollens and the plant height is less than 3 m so that it is easy to handle cross pollination.

Pollination and Embryo Rescue

Male flowers that are ready to be used are indicated by the crack of the pollen sac. Male flowers begin to break at 3:00-4:00 pm, and the next morning at 8:00-10:00 am the pollens are ready to pollinate to female flower, which is characterized by the opened bracts and formed angle 60°-90°.

The pollination was carried out at 8:00 am-01:00 pm. Receptive female flowers were characterized by the tip of the pistil head secreted gummy or slimy fluid at initially white colour, and followed by light yellow, yellow, blue and finally dark brown by means the pistil head was not receptive anymore. The pH of the liquid on the stigma can be measured with lacmus paper, if the pH showed 6 means the pistil was ready to receive pollen (<pH 6.0 and >pH 7.0, the pistil head was not receptive).

Out of approximately 13,000 pollinated fruits, 1.77% of them produced seeds. However, not all of the seeds contained embryos. The total 94 embryos were cultured in MS tissue culture medium added by 0.5 mgl-1 BAP, using method developed by [18] Afele and de Langhe (1991).

Not all of the embryos germinated, only 19 embryos developed, and finally only 3 embryos produced plantlets and coded as Kpk.02xJt.04, Kpk.04xJt.04 and Kpk.05xJt.04 respectively. Furthermore, the progenies were multiplied for further evaluation on the field.

Morphological Evaluation on The Field

Visually there were differences in the characters of KPK 05.04 compared to its parents, Barif 0025 and Barif 0130. Vegetatively growth characters which easily distinguished were the pseudostem colour and the wax layer on the stem. The pseudo stem colour of KPK 05.04 was shiny green with pink pigmentation. This character was a combination of its parent (Barif 0025 = pink pigmented opaque green and Barif 0130 = shiny green without pink pigment), while wax coating on pseudo stems and KPK leaf stalks 05.04 were less. this is the intermediary nature of the waxy Barif 0025 and the waxless Barif 0130. The proportion of KPK 05.04 peduncle was more likely to be similar to Barif 0130 and longer than Barif 0025.The male bud size of KPK 05.04 was bigger than this belong to both Barif 0025 and Barif 0130, while the bract imbrication of KPK 05.04 was slightly overlap which was also in between of the character belong to Barif 0025 and Barif 0130.

Even though the vegetative performance of plants showed the characters in between the two parents, fruit performance showed different things. The fruit size of KPK 05.04 was bigger than both parents. This occurred because of the heterosis phenomenon [19] Shull 1948; [20] Stansfield

1991; [21] Wardiana *et al*., 1995; [22] Tenkouano *et al*., 2003), where the superior characteristics of the parents were accumulated in the progenies.

Table 3. Heterosis effect and dominance level of controller gene of nine characters of KPK 05.04

No	Characters	Parents		KPK-05.04	Heterosis (%)	Heterobeltiosis (%)	h
		Barif-0025	Barif-0130				
1.	Bunch weight (kg)	1.47	11.33	14.47	126.09	27.71	1.64
2.	No. of fingers	71.77	94.33	88.67	6.77	-6.00	0.50
3.	No. of hands	5.38	7.73	6.67	1.75	-13.71	0.10
4.	Weight of finger (g)	13.56	110.01	148.22	53.68	34.73	1.79
5.	Max fruit weight (g)	20.09	144.00	172.22	109.91	19.60	1.46
6.	Min fruit weight (g)	5.72	70.67	114.44	199.62	61.94	2.35
7.	Flower bloom (days)	213.15	295.80	278.44	9.42	-5.87	0.58
8.	Days from flower bloom to harvest time (days)	108.08	93.40	107.89	7.10	-0.18	0.97
9.	Days from planting to harvest time (days)	321.23	389.20	386.33	8.76	-0.74	0.92
10.	Max fruit length (cm)	7.38	12.70	15.02	49.60	18.27	1.87
11.	Pedicle length (mm)	3.85	20.21	7.61	-36.74	-62.35	-0.54

From Table 3, it was revealed that effects of heterosis and heterobeltiosis of each character were found in the KPK 05.4 compared to the highest values belong to the parents. The genes actions found in the characters of KPK 05.04 were the imperfect positive dominant, imperfect negative dominant and overdominance. The over dominance characters were occurred in characters of bunch weight, fruit weight, maximum fruit weight, minimum fruit weight and maximum fruit length. The imperfect positive dominance characters were occurred in number of fruits, number of hands, flower bloom period, period from flower to harvest, and harvest time.

While the imperfect negative dominance was shown by the length of the fruit pedicel [23] (Sukartini *et al*., 2007).

The Evaluation of Plant Resistance Agains Fusarium Wilt

In order for new cultivar to be widespread to the community, the new cultivar must be registered and released by The Center for Plant Variety Protection and Agriculture Licensing (PVTPP), Ministry of Agriculture. Prior to register to PVTPP, a series of field evaluations must be carried out first, namely the superiority and the righteousness tests. The implementation of this evaluation was the planting of KPK 05.04 with other cultivars for comparison, on the field that has been infected by *Foc* wilt. From this evaluation plot, a number of information will be collected, namely resistance to *Foc*, the performance of morphological characters compared to other cultivars.

The result of field evaluation showed that none of the KPK 05.04 plants were infected by fusarium wilt, while the other cultivars, Barif 0025 and Ambon Hijau cultivars were infected by *Foc* with the disease severity were 3.33% and 23.3%, respectively [24] (Edison *et al*., 2017). Based on the results of this evaluation, KPK-05.04 was registered to be released under the name INA 03.

In September 2018, INA 03 was officially registered as a new banana cultivar with the Decree of the Ministry of Agriculture No. 072/Kpts/SR.120/D.2.7/6/2018.

Further Development

Prior to widely distribution, the new superior cultivar should be produced as breeder seeds and certified by The Seeds Inspection and Certification Institute (BPSB). Currently, the mother plants of INA 03 have been registered by BPSB as sources of suckers for further multiplication using tissue culture or conventional techniques. Some planting materials of INA 03 have been introduced to commercial tissue culture laboratory for further multiplication of extension seeds before distributed to the farmers. Extension seeds of INA 03 are also multiplying in ITFRI tissue culture laboratory for distributing to the farmers through the dissemination programs, especially the program of mitigating the treat of fusarium wilt.

Although the new banana cultivar that tolerant to *Foc* disease has been obtained, the breeding programs of banana are still being continued in order to produce the cultivars with several superior characters such as, tolerant to other diseases (blood disease, banana bunchy top virus, sigatoka leaf streak, etc.), better quality for dessert or for cooking consumption, longer self-life and better fruit performance.

Table 3. The comparison of morphological characters of INA 03, Barif 0025 dan Barif 0130

No	Barif 0025	INA 03	Barif 0130
1. Plant performance			
			
2. Waxiness			
			
3. Bunch orientation			
			

4. Male bud apex

Fig. 1. The comparison of ripe fruits of INA 03, Barif 0025 dan Barif 0130

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The Effectiveness and Bacterial Communities of Biofertilizer Application for Huanglongbing Disease Control in Indonesia

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Abstract

Huanglongbing (HLB) which is caused by *Candidatus* liberibacter asiaticus (*C*Las) is one of the citrus diseases that cause the largest losses on citrus orchards in Indonesia and other countries. An experiment was conducted at the HLB endemic area in Purworejo district of Central Java to study the response of biofertilizer treatment on HLB susceptible citrus plant (*Citrus reticulata*) against the disease. The trial was using graft inoculated and non-inoculated by inoculum HLB.

Plants treated by biofertilizer A (manufactured) or biofertilizer B (farmer's formulation), once a month during 3 years and 8 months. *C*Las detection was conducted in the last 9 months. The results showed that application of biofertilizer B was effective in reducing HLB disease naturally transmitted by vector (AUDPC value of 195.24 by leaf score and 218.58 by canopy score).

Biofertilizer A was effective to control HLB disease on HLB-inoculated plants. Detection of *C*Las by PCR (Polymerase Chain Reaction) show that some plants have instability in the detection results that were influenced by the concentration of *C*Las. The rhizobacteria population in plant without the HLB inoculation and applied biofertilizer A were higher than in plants with the HLB inoculation and applied biofertilizer B. The total population of rhizobacteria tended to increase except in the treatment of biofertilizer A, that were applied on HLB-inoculated plants. The application of biofertilizer to artificially infected plants and naturally transmitted through vectors were able to prolong the viability of the plant.

Keywords: AUDPC, biofertilizer, huanglongbing, population, rhizobacteria

Introduction

Huanglongbing (HLB) or Citrus Vein Phloem Degeneration (CVPD) is one of the citrus diseases which has an impact in decreasing citrus production. The disease is caused by bacterium *Candidatus liberibacter asiaticus* (*C*Las) which transmitted by *Diaphorina citri* Kuwayama vector [1], [2]. The infected plants do not carry out its physiological functions according to the genetic potential.

The productivity of infected plants rapidly decreases without control measures. Some HLB control techniques have been performed but the results have not been satisfactory. Management of diseases with nutritional approaches, use of soil enhancers, intercropping with guava, antibiotic, hot temperatures and other measures have been taken to control the pathogens and their vectors [3], [4], [5], [6], [7].

Citrus plants have microbes that are beneficial to plants and there are also harmful ones (pathogens). Pathogens affect other microbial diversity qualitatively and quantitatively. According to [8] there was a difference of microbial composition between healthy plant roots and infected by *C*Las based on results of bacterial clone sequencing. [9] suggested the microbial community in the *C*Las infected plant rhizosphere has shifted from the use of a more degradable carbon source to a more stable form. Changes in plant physiology which is caused by *C*Las infection can lead to a shift in the functional potency and composition of the rhizosphere microbial community. The diversity of bacteria in HLB infected plants is influenced by the treatment such as the addition of antibiotics. Generally, the infected plants occur a decrease in the diversity of *C*Las bacteria.

Bacteria including α-proteobacteria present at the lowest level of treatment with antibiotics [10]. The application of Fe in HLB infected plants also affects the *C*Las ecosystem, thus the bacteria become undetectable after treatment in some plants [11].

The effect of biofertilizer has not much studied in the HLB-disease management. Biofertilizer is an active biological product consisting of microbes that can improve fertilizer efficiency, fertility and soil health (70/Permentan/Sr.140/10/2011). Biofertilizers are products that contain life microorganisms, which were directly or indirectly affect the growth of plants and crops through different mechanisms. Biodegradable limits may include products containing bacteria to control pathogens [12],[13]. The aims of this research were to study the response of biofertilizer treatment on HLB susceptible citrus plant (*Citrus reticulata*) against the disease.

Material and Methods

The research was conducted at citrus orchard in Purworejo, Central Java, Indonesia. Siam (*Citrus reticulata*) cultivar seedling was used as the research material. Seedling were inoculated with bark tissue from infected plants. Two biofertilizers were applied on citrus plants from the beginning of planting until 3 years and 8 months i.e., manufactured biofertilizer (biofertilizer A) and biofertilizer made by farmers (biofertilizer B). Biofertilizer B contains cattle rumen, coconut water, rice water, banana tuber and EM 4. Observations were done after two years of treatments.

The negative control plants were plants without both inoculation of pathogen and application of biofertilizer, while the positive controls were inoculated plants but without application of biofertilizer. Each treatment was used 10 plants as replicate. Amount of 60 plants were treated.

Observations were made on the intensity of the disease, detection of the presence of bacteria and bacterial community profile of the biofertilizer and plant rhizosphere by PCR (*Polymerase Chain Reactions*).

1. Huanglongbing disease intensity

Disease intensity was observed based on the severity using a score of the symptoms appear on leaves and canopies. Symptoms observed were included chlorosis, reduction leaf size and typically restriction to the terminal shoots, and blotchy mottle, which was visible on terminal shoots as well as on the proximal parts of branches [14]. Observations were made on each tree for every treatment before 3 years of the treatments up to 8 months after that. Disease intensity was calculated by disease intensity formula [15].

The calculation of the disease intensity was used to measure the percentage of disease inhibition based on the value of Area Under Disease Progress Curve (AUDPC). The value of AUDPC in each treatment was obtained by AUDPC formula [16].

2. Detection of HLB disease using PCR methods

a. DNA extraction

Six to ten leaves per tree for each treatment were collected from the field. The total DNA was extracted from 0.3 g midrib of leaves sample by using modified CTAB buffer method [17].

Samples were macerated and put it into Eppendorf tube which was contained of 1500 µl buffer of DNA isolation + 30 µl mercapthoethanol, mixed it and incubated at 65 ºC for 15 minutes. After that, the mixture was centrifugated at 6000 pm for 5 minutes in the micro centrifuge. A ± 750 µl supernatant was transferred to new tube then added with chloroform solution: Isoamyl alcohol (24:1), equal with the supernatant (750 µl) to separate DNA solutions from another materials, then mixed well and centrifuged at 12000 pm for 10 minutes. 600 µl of the supernatant was taken, added with cold isopropanol as much as the supernatant, mixed well then continued with centrifugation at 13000 rpm for 10 minutes. DNA pellet was washed with cold 70% alcohol then centrifuged at 10000 rpm for 3 minutes and dried in the laminar air flow. Then, 20-40 µl buffer TE was added in to DNA pellet and stored at -20 °C.

b. DNA amplification, separation and visualization

Amplification of *C*Las DNA was done using primer pairs Las606 (5 GTG AGT GAG-GGA GGA ATT CCG A-3) and LSS (5-ACC CAA CAT CTA GGT AAA AAC C -3) [18], [19]. PCR mix composition for 25µl reactions volume/sample: 12.5µl of Kapa 2G Fast ReadyMix PCR kit (Kapa Biosystems), 2µl of each primer, 6.5µl of H_2O, and 2µl of DNA extracts. The PCR (Biorad machine) profile conditions were 96 °C for 9 min, followed by 35 cycles of 96 °C for 30 s, 55 °C for 30 s, 72 ºC for 60 s, and followed by the final extension at 72 °C for 7 min [19]. PCR product (DNA) were separated using electrophoresis method on 1 % agarose gel (Sigma) contain ethidium bromide (10 mg l^{-1}) in 1 x TBE solution. DNA electrophoresis was done using Mini sub-cell system (Bio-Rad Laboratories, USA) at 75 volts for 50 minutes. DNA bands were observed with biodoc gel (Biorad). Presence of the predicted 500-bp 16S rDNA band indicate in the *C*Las positive samples and no band indicate in the *C*Las negative sample.

3. Bacterial community profile from plant rhizosphere by PCR method

a. Soil rhizosphere bacteria extraction and isolation

Isolation of bacteria from soil rhizosphere area used 1 g of soil that was dissolved in 10 ml water then made series dilution (10^{-2}-10^{-4}). The solution was grown on Nutrient Agar medium by spread-plating method to obtain cultured cells as colony forming units (CFU). Multiplication of bacteria in a specific dilution series used 3 replications, it was incubated at 26 °C for 48 h, and followed by observing DNA population. The density of the bacterial cell was shown as a dry weight CFU g^{-1} log. The leaves were cleaned from the soil particles prior to the isolation of bacteria in the leaf midribs. Before the bacteria were isolated, leaf midribs were washed sequentially as follows: washed with tap water, immersed in 70 % ethanol for 3 minutes, washes with 2.5% Clorox for 5 minutes, rinsed with 70% ethanol for 30 seconds and washed 3 times with sterile water. The leaf midribs were cut into pieces, then crushed and enriched with PBS-peptone (PBS-pepton 22.5 ml plus 2.5 g vein leaves). The crushed leaf midribs in PBS-pepton were homogenized with a stirrer for 5 minutes. 100µl bacterial suspension was spread-plating with L glass on Nutrient Agar (NA) medium. Observation of colony of populations was done 48 hours later.

The extraction of bacterial mixture was done by SDS method. The bacteria on the NA medium are harvested, inserted pellets into 1.5µl microtube then centrifugated at 6000 rpm. Bacterial solution was added with 570µl TE, then mixed by a vortex, followed by added 50µl with 10% SDS, and incubated 1 hour at 37 °C. It was added with 100µl 5 M NaCl and mixed, then added with 80µl CTAB/NaCl solution. The suspension was mixed and incubated for 10 minutes at 65 °C if it was not well dissolved. The sample solution was supplemented with 700µl CIAA (Chloroform Isoamil Alcohol/24:1), mixed and centrifuged at 12000 rpm for 5 min. The supernatant solution (± 900µl) was placed in a new microtube, while the pellet was discarded, then centrifugated at 6000 rpm.

The supernatant was supplemented with PCIAA (Phenol, Chloroform Isoamil Alcohol /25:24:1) amount of supernatant volume (± 1000-1500µl), then centrifuged at 12000 rpm for 5 min. The supernatant was taken (± 650µl) and placed on a new microtube, then added with isopropanol (± 300µl), shaken gently then centrifuged at 12000 rpm for 5 min. The solution was discharged, the pellets washed with 70% cold ethanol (500µl), centrifuged 12000 rpm for 5 min.

The supernatant removed carefully, then the pellet dried in a laminar air flow. The dry DNA pellet was added with 20-40µl TE buffer and stored at -20 °C.

b. DNA amplification, separation and visualization

DNA amplified using the gene region between 16S rDNA-23S rDNA (Ribosomal Intergenic Spacer Analysis) from bacteria, S926f (5-TAC GHY THT RTT-3), L189r (5- CTY AAA KGA ATT GAC GG-3) [20]. Composition of PCR reaction for 25 reactions/samples as follows: 12.5µl PCR kit (Kapa2G Fast ReadyMix), 1.25µl each primer, 2µl DNA, 8µl dd H2O. The DNA amplification profile was used as follows: 1 cycle of pre denaturation at 95 °C for 2 minutes, 35 cycles (denaturation at 94 °C for 15 seconds, annealing at 47 °C for 15 seconds, extension at 72 °C for 2 min) and 1 final extension cycle at 72 °C for 5 minutes. The amplified DNA product was separated by electrophoresis method in 1.5% agarose gel in a 1 x TBE solution for 80 min at a 60-volt voltage, then immersed separately in ethidium bromide (10 mg l-1) as a dye for 15 minutes.

5µl of 1 Kb ladder DNA was used, DNA band detection was done by UV transilluminator.

Results

1. Disease intensity development based on visual symptoms and Area Under Disease Progress Curve

The intensity of HLB disease based on symptoms on leaf and canopy tended to show similarity of results although the calculation of disease intensity based on canopy score was higher than based on the leaf score at the beginning of the treatment (Fig. 1). Application of biofertilizer B caused a decrease in the intensity of HLB disease after treatment.

The effectiveness value of control calculated based on the development of disease intensity showed that the application of biofertilizer B was most effective in reducing HLB disease with the smallest AUDPC value of 195.24 (based on leaf score) and 218.58 (based on the score of the canopy) (Fig. 2).

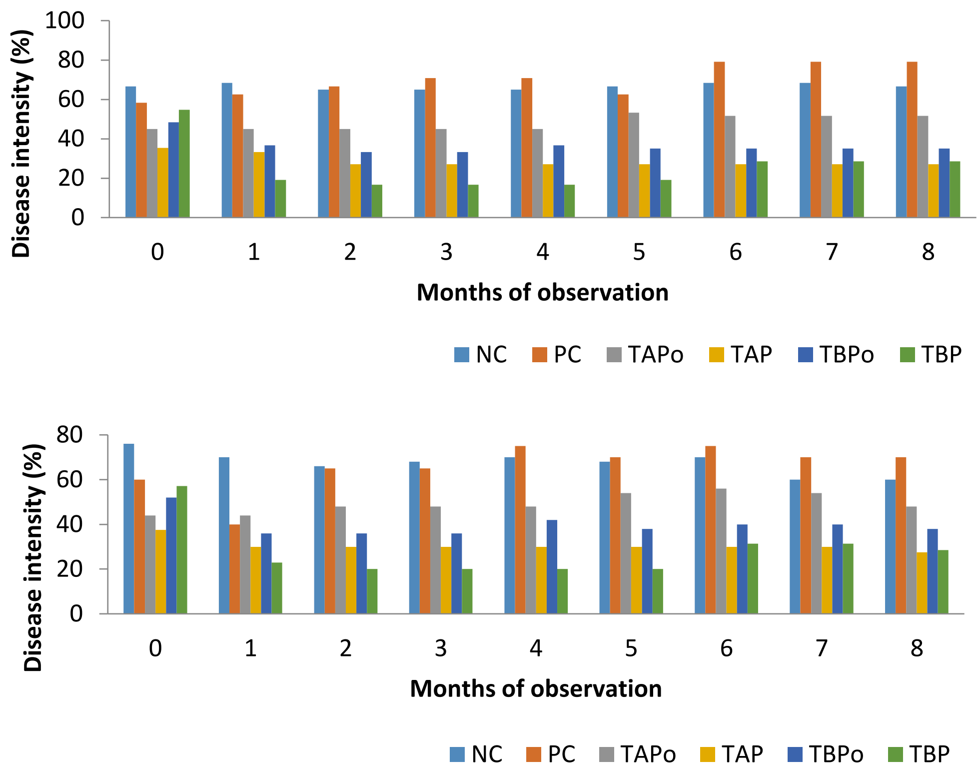

Fig. 1. Disease intensity (%) based on score of symptoms on leaf (top) and canopy (bottom)
Note: NC: Negative control; PC: Positive control; TAPo: Application of biofertilizer A without HLB inoculation on plant; TAP: Application of biofertilizer A with HLB inoculation on plant; TBPo: Application of biofertilizer B without HLB inoculation on plant; TBP: Application of biofertilizer B with HLB inoculation on plant

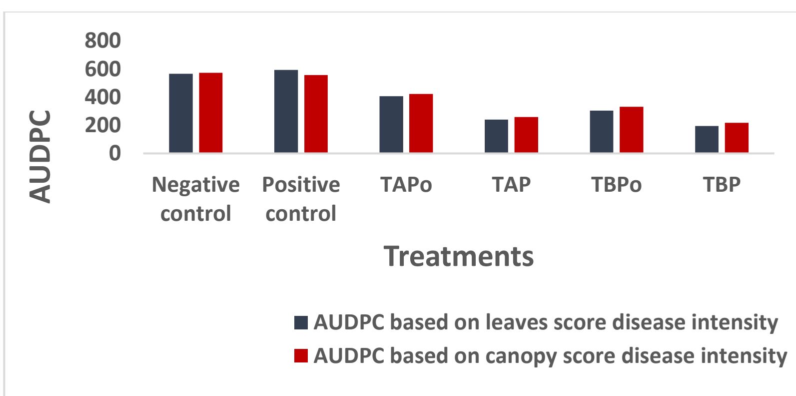

Fig. 2. Area Under Disease Progress Curve (AUDPC) value on each treatment
Note: TAPo: Application of biofertilizer A without HLB inoculation on plant; TAP: Application of biofertilizer A with HLB inoculation on plant; TBPo: Application of biofertilizer B without HLB inoculation on plant; TBP: Application of biofertilizer B with HLB inoculation on plant

2. Plants mortality and presence Candidatus liberibacter asiaticus

Plants mortality due to pathogen infection was started at two years after treatments. Plants condition until three years treatments were showed on Table 1. Plants without artificial inoculation of HLB (NC and TAPo) showed more plants infected with HLB disease than plants with HLB inoculation (PC and TAP). This is due to the transmission of HLB disease through vectors from infected plants at others citrus orchards. Purworejo district is one of endemic areas of HLB disease in Indonesia. This area is located at an altitude of 60 m ASL, which has the appropriate temperature and humidity conditions for the development of HLB vectors [21].

Biofertilizer B (farmer-made) applied to plants with/without artificial inoculation was more able to maintain plant health. According to [22] the fingerprint of biofertilizer showed considerable variation based on BOX-PCR and ERIC-PCR techniques, while bacteria from biofertilizer A have were fewer variable bands based on three PCR techniques (BOX-PCR, ERIC-PCR and REP-PCR).

Table 1. Percentage plant mortality after treatments and presence of *Candidatus liberibacter asiaticus*

Treatments	Plant conditions (%)				Presence of CLas bacteria (%)				
	Healthy plants	Infected plants	Plant death at 24 months	Plant death after 36 months	24 months	27 months	30 months	33 months	36 months
NC	0	70	0	30	*50/0	50/0	40/0	90/0	80/0
PC	0	30	60	10	30/60	10/60	20/60	10/60	30/60
TAPo	30	70	0	0	30/0	50/0	0/0	60/0	50/0
TAP	30	50	20	0	10/20	10/20	0/20	50/20	30/20
TBPo	60	20	0	20	30/0	20/0	10/0	20/0	30/0
TBP	50	20	30	0	0/30	10/30	0/30	0/30	20/30

*Note: NC: Negative control; PC: Positive control; TAPo: Application of biofertilizer A without HLB inoculation on plant; TAP: Application of biofertilizer A with HLB inoculation on plant; TBPo: Application of biofertilizer B without HLB inoculation on plant; TBP: Application of biofertilizer B with HLB inoculation on plant, * presence of CLas/plant mortality (%).*

Molecular detection of HLB showed that some plants have instability in the detection results with conventional PCR. Some plant was positively infected with HLB but on the next observation did not infected or vice versa, although conventional PCR analyses used Las606 and LSS primers pairs that were 10 times higher in sensitivity than using primers that first assembled to detect HLB disease i.e., OI1 and OI2c [19]. A dynamic concentration of *C*Las pathogens in plants affects the detection results with conventional PCR. The dynamics of *C*Las pathogen concentration is influenced by the severity of symptoms and seasons. In this study, observations that were done before treatment, 9 months treatment and 12 months treatment, were in the rainy season, while observations on 3 months treatment and 6 months treatment were in the dry season. There was a tendency of higher *C*Las cell density in dry season so that PCR detection also shows infected plants with mild symptoms. The negative control plants showed a tendency to become infected based on detection by conventional PCR, although it did not cause death until the 3-year-old plant.

The percentage of plant deaths in this treatment is highest compared to other treatments after three years old. Inoculated plants without biofertilizers treatments that still survive showed that most of them were infected by *C*Las. Many plants experience was death during treatment both without application of biofertilizers and without artificial transmission. The application of biofertilizer on infected plants was able to prolong the viability and the productivity of plant. The productivity of infected plant can be maintained although cell density does not show a consistent reduction due to treatments.

3. The bacterial community of plant rhizosphere

The rhizobacteria population in plant without HLB inoculation and applied biofertilizer A was higher than in plants with HLB inoculation and applied biofertilizer B after 3 years and 9 months application (Fig. 3). The population of rhizosphere bacteria on biofertilizer application was higher than the control. The application of biofertilizer to HLB-infected plants act as induce systemic resistance (ISR) that will enrich the diversity of microorganisms in the rhizosphere which it will protect the plant from infection and reduce the development of HLB disease.

Total population of rhizobacteria in all treatments tended to increase at 9 months of treatment except total rhizobacteria on HLB-infected plants which applied by biofertilizer A. This suggest that biofertilizers A did not able to influence increasing of rhizobacterial populations in HLB-infected plants. The composition of microbial communities in the rhizosphere is related to nutrient diversity and secondary metabolites in root exudates [23]. HLB infection has an effect on plant nutrient content, especially micro nutrients. According to [9] pathogens can alter the structure of different native microbial communities between rhizobacteria from HLB-infected plants and healthy plants, although there is no a direct competition effect. The rhizobacterial population after 6 months without biofertilizer treatments showed a few bacterial diversities in each treatment (only about 3-5 bacterial groups each treatment). The diversity of bacteria is very small because isolation of bacteria with standard culture media is only able to isolate 0.1% of the total bacteria present in the soil [24]. The length of the DNA bands obtained by RISA primers in this study ranged from 250-4000 bp. Differences in the presence of DNA bands on electrophoresis results show differences in the diversity of bacterial populations in the rhizosphere. DNA bands of a certain thickness indicate the presence of abundant rhizosphere bacteria in a sample [25].

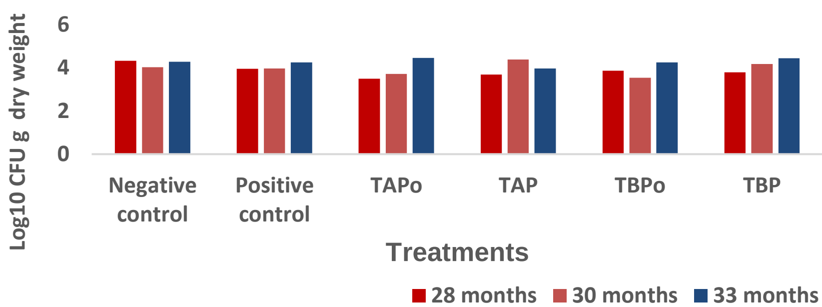

Fig. 3. Rhizobacteria structural population on biofertilizer application

Note: TAPo: Application of biofertilizer A without HLB inoculation on plant; TAP: Application of biofertilizer A with HLB inoculation on plant; TBPo: Application of biofertilizer B without HLB inoculation on plant; TBP: Application of biofertilizer B with HLB inoculation on plant.

Rhizobacterial diversity at 28 months treatment was higher than at 30 months treatment (Figure not showed). It was influenced by soil, plant water content and bacterial cell density in the roots.

Condition at 28 months treatment was the end of the dry season, while at 30 months treatment was the beginning of the rainy season. In addition, changes in the presence DNA bands on electrophoresis results indicate that HLB infection and fertilization treatment by biofertilizer affect root exudates produced by plants that was affect the rhizobacterial community. As a result, fertilization by biofertilizer on infected plants can suppress the development of HLB disease. The community of rhizosphere bacteria in plants which applied by biofertilizer A was more diverse than was biofertilizer B at 28 months treatment. It is assumed that cow rumen on biofertilizer A contains many enzymes that was play a role in the development and diversity of bacteria.

Rhizobacteria derived from positive control plants show the same diversity with rhizobacteria from HLB-infected plants both through artificial inoculation or natural transmission through vector which applied by biofertilizer A. This indicates that the similarity of the rhizobacterial community diversity is due to all treatments contain plants infected. The addition of biofertilizer A has no effect on the rhizobacterial diversity on HLB-infected plants. Application of biofertilizer

B on non-inoculated plants although naturally infected HLB by vector shows a different pattern of DNA bands. The inoculation of *C*Las bacteria affects to the diversity of rhizobacteria.

Conclusions

The effectiveness value of control showed that the application of biofertilizer which made by farmer was most effective in reducing HLB disease.

The type of biofertilizer effects the rhizobacteria population. The community of rhizosphere bacteria in plants which applied by biofertilizer A (made by farmers) was more diverse than was biofertilizer B (made by manufactory).

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Assessment of Variability, Heritability and Divergence of Ciplukan [Cutleaf Ground Cherry: (*Physalis angulata* L.)] to Increase Exotic Fruit Genetic Capacity in Indonesia

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Abstract

Genetic variability and heritability of crop characteristics are important drivers for crop improvement. Genotypic divergence is the basis for an adequate phenotypic selection. The objectives of the study were to estimate phenotypic and genetic coefficients of variation, heritability in broad sense, genetic advance and grouping genotypes. A field experiment was conducted in Areng-Areng, Junrejo, Batu City, East Java during the period May to September 2017. Different plant characteristics of 24 cut leaf ground cherry or ciplukan (*Physalis angulata* L.) genotypes were studied to assess morphological variability, heritability, and genetic divergence. Analysis of variance revealed that there were significant differences among characters of genotypes with exception of fruit acidity. The 24 genotypes studied were high phenotypic and genotypic coefficient of variation for most characteristics. High heritability's were found on plant height at first branching, number of tertiary branches, number of flowers per plant, number of fruits per plant, calyx length, average individual fruit weight with calyx, average individual fruit weight without calyx, fruit total soluble solids, fruit weight with calyx per plant, and fruit weight without calyx per plant. It can be considered as of great importance for improving cut leaf ground through phenotypic selection. Most characters show a high genetic advanced. A dendrogram showed that the 24 genotypes were grouped into three groups. Because of their specific characteristics, the genotypes in each group can be used as material for recombination to improve cut leaf ground cherry or ciplukan cultivars.

Keywords: Physalis angulata*, genetic parameters, plant breeding, plant genetic resources*

Introduction

Physalis angulata L is well-known under the common name of cut leaf ground cherry. This species is distributed throughout the archipelago in Indonesia [1-10] giving the potential biological genetic resources of this species the chance to be developed. Although the general spread over the archipelago there is no known common name in Indonesia. Cut leaf ground cherry has various regional names, namely *leletop, daun boba, daun kapo-kapo, daun lato-lato, leletep, depuk-depuk* (Sumatera), *cecendet, ciplukan* (Jawa), *leletopan, leletokan, daun kopo-kopo, daun loto-loto, lupareho, valanpanga, kateo-teo, roiye, toto* (Sulawesi), *karuhux, antokop, daun leletup* (Kalimantan), *angket, kepok-kepokan, keceplokan* (Bali), *daun boba, lapinonat, lapunonat,* *dagameme* (Maluku), *nyornyoran, yoryoran*, (Madura), *kaciputan* (Bawean), *kenampokan, dedes* (Lombok), *telak* (Flores) and *kakuto, gekatomato* (Papua).

The different environments (e.g., altitudes) and the isolation of plant populations due to the distance between islands create a large genetic variability [11]. The variety of local names and culture-based utilization can be used as clues to the genetic potential of abundant local germplasm that needs to be conserved and utilized. Cultivation status of cut leaf ground cherry or *ciplukan* is still considered as a wild species [1, 2] and is seeing as a weed [12-14] that interferes with the growth of cultivated plants. Nevertheless, the community uses as a raw material for traditional medicine [4, 15]. Empirically these plants have beneficial compounds for humans that are related to healing cancer [16-18], diabètes [19], inflammation [20], and malnutrition [21].

Cut leaf ground cherry fruit is very popular and can be used as an exotic fruit for direct consumption. Cut leaf ground cherry fruit has a relatively small size inside calyx, has a sweet and sour taste. The fruits have a high antioxidant, vitamin and mineral content [22-24]. Besides that, the fruit contains active compounds related to anti-diabetic activity, anti-microbial activity, anti-oxidant and cytotoxic activities [23, 25], postpartum infections, diuretic, asthma, malaria, inflammations, scabies [18]. Thus, cut leaf ground cherry fruit is a valuable source for fresh food, nutraceutical, and pharmaceutical industries.

The genetic potential of fruit characteristics of cut leaf ground cherry can be increased through recombination and selection in plant breeding programs. Assessing genetic parameters as variability, heritability, and the divergence through a quantitative genetic approach [26-28] is of great importance for plant breeding programs. The objectives of the study were to estimate phenotypic and genotypic coefficients of variation, heritability in broad sense, genetic advance and grouping genotypes as a basis of divergence for selection to increase cut leaf ground cherry genetic capacity.

Materials and Methods

Twenty-four genotypes were followed up in under field conditions in Areng-Areng (635 m asl), Junrejo, Batu City, East Java during the period May to September 2017. Information about the genotypes studied are shown in Table 1. Cut leaf ground cherry seeds germinated in plastic trays filled with organic growing medium. Thirty-day-old bedding plants were transplanted to the field.

Genotypes were evaluated in a completely randomized block design with three replications. Plots comprised of a single row of 2.5 m long. Between and within plot spacing was 0.80 m and 0.40 m, respectively. On 5 plants in each plot 16 characteristics were monitored. The characteristics evaluated in this study were plant height at first branching, stem diameter, number of tertiary branches, number of flowers per plant, number of fruits per plant, peduncle length, calyx length, calyx diameter, fruit diameter, fruit length, average individual fruit weight with calyx, average individual fruit weight without calyx, fruit total soluble solids, fruit acidity, fruit weight with calyx per plant, and fruit weight without calyx per plant. Analysis of variance was carried out on the data to test significance differences [29], and estimated phenotypic and genotypic variances and their coefficients of variation, heritability in a broad sense and the genetic advance for each character [30] as follow:

$$\text{Genetic variance } (\sigma_g^2) = \frac{(\sigma_e^2 + r\sigma_g^2) - \sigma_e^2}{r}$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \sigma_e^2$$

$$\text{Genetic coefficient of variation (GCV)} = \frac{\sqrt{\sigma_g^2}}{\bar{\bar{X}}} \times 100\%$$

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma_p^2}}{\bar{\bar{X}}} \times 100\%$$

GCV and PCV were high if >25%, moderate if between 10%-25%, and low if <10% [31].

$$\text{Broad sense heritability (H)} = \frac{\sigma_g^2}{\sigma_p^2} \times 100\%$$

Heritability high if H>50%, moderate if 20%≤H≤50%, and low if H<20% [32].

$$\text{Genetic advance (GA)} = iH\sigma_p$$

$$\text{Genetic advance as percent of mean (GAM)} = \frac{iH\sigma_p}{\bar{\bar{X}}} \times 100\%.$$

GAM categorized as low (<10%), moderate (10-20%) and high (>20%)[33].

where:

σ_p = phenotypic standard deviation

i = selection differential (2.06 at 5% selection intensity)

$\bar{\bar{X}}$ = grand mean of the characters

A dendrogram was performed for grouping genotypes as a basis of divergence generated through agglomerative hierarchical clustering based on agglomeration method unweighted pair-group average and the similarity Pearson correlation coefficient. Analysis of data was done by using Microsoft® Excel 2010/XLSTAT version 2014.5.03.

Table 1. Genotypes of cut leaf ground cherry

Genotype code	Origin	Pedigree
GRT-DK-B	Garut, West Java	Pure line selection method from landrace
GRT-KL-B	Garut, West Java	Pure line selection method from landrace
KDR-NL	Kediri, East Java	Pure line selection method from landrace
KDR-RM (02)	Kediri, East Java	Pure line selection method from landrace
MLG-JK (01)	Malang, East Java	Pure line selection method from landrace
MLG-LW (01)	Malang, East Java	Pure line selection method from landrace
MLG-LW (07)	Malang, East Java	Pure line selection method from landrace
MLG-LW (09)	Malang, East Java	Pure line selection method from landrace
MLG-LW (10)	Malang, East Java	Pure line selection method from landrace
MLG-TP (01)	Malang, East Java	Pure line selection method from landrace
MLG-TP (02)	Malang, East Java	Pure line selection method from landrace
MLG-TP (04)	Malang, East Java	Pure line selection method from landrace
MLG-TP (05)	Malang, East Java	Pure line selection method from landrace
SPG-AS (01)	Sampang, Madura, East Java	Pure line selection method from landrace
SPG-AS (02)	Sampang, Madura, East Java	Pure line selection method from landrace
SPG-AS (04)	Sampang, Madura, East Java	Pure line selection method from landrace
SPG-DC	Sampang, Madura, East Java	Pure line selection method from landrace

SPG-GD	Sampang, Madura, East Java	Pure line selection method from landrace
TLG-PL (02)	Tulungagung, East Java	Pure line selection method from landrace
TLG-PL (02-02)	Tulungagung, East Java	Pure line selection method from landrace
TLG-PL (07)	Tulungagung, East Java	Pure line selection method from landrace
TSK-IH(B-01)	Tasikmalaya, West Java	Pure line selection method from landrace
TSK-IH(B-02)	Tasikmalaya, West Java	Pure line selection method from landrace
UNK (12)	Unknown	Pure line selection method from landrace

Results and Discussion

The analysis of variance revealed significant differences among the cut leaf ground cherry genotypes for all characteristics with exception for fruit acidity (Table 2). The level of variation indicates the presence of sufficient variability among the evaluated cut leaf ground cherry genotypes to set up a successful breeding program. It reflects the existing of large variability among genotypes and this variability can be further utilized in the cut leaf ground cherry plant breeding programs.

Table 2. Mean square 16 characters 24 cut leaf ground cherry genotypes

Characters	Means Square	Prob	Sign
Plant height at first branching (cm)	7.20	0.00	**
Stem diameter (cm)	0.14	0.00	**
Number of tertiary branches	304.80	0.00	**
Number of flowers per plant	7125.09	0.00	**
Number of fruits per plant	6440.31	0.00	**
Peduncle length (cm)	0.05	0.00	**
Calyx length (cm)	0.28	0.00	**
Calyx diameter (cm)	0.05	0.00	**
Fruit diameter (cm)	0.03	0.01	**
Fruit length (cm)	0.08	0.00	**
Average individual fruit weight with calyx	0.27	0.00	**
Average individual fruit weight without calyx	0.23	0.00	**
Fruit total soluble solids (TSS) (°Brix)	4.86	0.00	**
Fruit acidity (pH)	0.13	0.41	
Fruit weight with calyx per plant (g)	17939.90	0.00	**
Fruit weight without calyx per plant (g)	0.13	0.00	**

*Prob = probability; * and ** significance level at 0.05 and 0.01 of probability respectively*

Estimation of genetic parameters consisting of phenotypic variances, genetic variances, genetic coefficients of variation (GCV), phenotypic coefficients of variation (PCV), heritability, genetic advance, and genetic advance as percent of the mean are given in Table 3. Genetic variance values ranged from 0.003 for fruit acidity to 5498.68 in fruit weight with calyx per plant while phenotypic variance values ranged from 0.022 for fruit diameter to 6942.54 for number of fruits per plant. The GCV values were ranged from 1.42% for fruit acidity to 60.45% fruit weight without calyx per plant. Similarly, the PCV values ranged from 8.83 in peduncle length to 66.85 in fruit weight without calyx per plant. GCV and PCV in all characters were categorized into low, moderate, and high. There were characteristics of the cut leaf ground cherry that have the same variability level based on GCV and PCV values. Number of tertiary branches, number of flowers per plant, number of fruits per plant, fruit weight with calyx per plant, and fruit weight without calyx per plant have the same criteria for PCV and GCV, which is high variability. Stem diameter has high criteria for

PCV while GCV is moderate. Moderate variability based on PCV and GCV values is found in the characters plant height at first branching, calyx length, average individual fruit weight with calyx, and average individual fruit weight without calyx. In some characters, it was identified that variability was caused by phenotypic factors showed moderate but genetic factors showed low variability. Different of PCV and GCV criteria were observed in fruit diameter, fruit length, and fruit total soluble solids. Characters that consistently show low PCV and GCV were peduncle length, calyx diameter, and fruit acidity. GCV provide information on genetic variability present in the population. Same criteria high variability on GCV and PCV indicated the existence of substantial variability for such characteristics. Selection may be effective because variations among the genotypes were mostly due to genetic factors. Individual plant selection can be practiced for the characters to get better criteria in traits. If on a character that will increase its genetic capacity has a greater PCV value than GCV then it must be considered about how the new population will be formed and determine the right selection method so that the character can be inherited and identified its superiority, and must be convinced that the character it is controlled by genetic factors, not by non-genetic factors.

Heritability values are helpful in predicting the selection progress through the selection process.

Broad-sense heritability in cut leaf ground cherry character ranged from 2.35% for fruit acidity to 92.64% for fruit total soluble solids (Table 3). Broad-sense heritability is categorized as low (H<20%), moderate (20≤H≤50%) and high (H>50%), [32]. Plant height at first branching, number of tertiary branches, number of flowers per plant, number of fruits per plant, average of calyx length, average of individual fruit weight with calyx, average of individual fruit weight without calyx, fruit total soluble solids, fruit weight with calyx per plant, fruit weight without calyx per plant have a high heritability. These traits were controlled by a few genes and are less influenced by the environment.

Table 3. Estimation of genetic parameters 16 characters in 24 cut leaf ground cherry genotypes

Characters	σ^2_g	σ^2_p	GCV(%)	PCV(%)	H(%)	GA	GAM(%)
Plant height at first branching (cm)	2.01	3.17	14.07	17.64	63.62	2.33	23.12
Stem diameter (cm)	3.13	8.08	19.33	31.08	38.68	2.27	24.77
Number of tertier branchs	98.43	107.93	31.50	32.98	91.20	19.52	61.97
Number of flowers per plant	1979.28	3166.54	39.26	49.66	62.51	72.46	63.94
Number of fruits per plant	1998.16	2443.99	51.55	57.01	81.76	83.26	96.02
Peduncle length (cm)	0.01	0.03	5.68	8.83	41.36	0.14	7.53
Calyx length (cm)	0.08	0.12	12.05	15.26	62.42	0.45	19.62
Calyx diameter (cm)	0.01	0.03	6.65	9.65	47.58	1.55	9.46
Fruit diameter (cm)	0.01	0.02	5.62	10.35	29.53	0.90	6.29
Fruit length (cm)	0.02	0.04	8.27	12.27	45.46	0.19	11.49
Average fruit weight with calyx	0.09	0.10	16.03	17.04	88.43	0.57	31.04
Average fruit weight without calyx	0.07	0.08	16.13	17.15	88.37	0.52	31.23
Fruit total soluble solids (TSS) (°Brix)	1.58	1.71	9.85	10.24	92.46	2.49	19.50
Fruit acidity (pH)	0.00	0.13	1.42	9.30	2.35	0.02	0.45
Fruit weight with calyx per plant (g)	5498.68	6942.54	56.82	63.84	79.20	135.95	104.16
Fruit weight without calyx per plant (g)	5209.00	6371.08	60.45	66.85	81.76	134.44	112.60

Note: σ^2_g = genetic variance, σ^2_p = phenotypic variance, GCV and PCV criteria: low (<10%), moderate (10%-25%), high (>25%). H= broad sense heritability. H is categorized low (H<20%), moderate (20≤H≤50%), high (H>50%). GA = genetic advance, GAM = genetic advance as percent of mean. GAM is categorized as low (<10%), moderate (10-20%) and high (>20%).

Knowledge of heritability together with genetic advance (GA) or is more useful. GA referred to the improvement of characteristics in genetic value for the new population compared with the population of origin at given selection intensity; it is important to predicting the expected genetic gain from one cycle of selection. Representation from GA is genetic advance as percent of the mean (GAM). Plant height at first branching, stem diameter, number of tertiary branches, number of flowers per plant, number of fruits per plant, average of individual fruit weight with calyx, average of individual fruit weight without calyx, fruit weight with calyx per plant, fruit weight without calyx per plant has high genetic advance.

Selection as an effort to increase genetic capacity in a characteristic will succeed if the population consisting of potential genotypes has high phenotypic and genetic variability with high heritability and is supported by high genetic advance. Based on the genetic parameter category, the characters number of tertiary branches, number of flowers per plant, number of fruits per plant, fruit weight with calyx per plant, and fruit weight without calyx per plant can be used as a reference to improve the genetic capacity of cut leaf ground cherry through breeding. Based on these selected characteristics in cut leaf ground cherry, it can be seen that the criteria for heritability are not always linear with measures of genetic variability and genetic advance. A high heritability does not always reflect high genetic and phenotype variability, although high heritability almost always shows high genetic advance (Table 3).

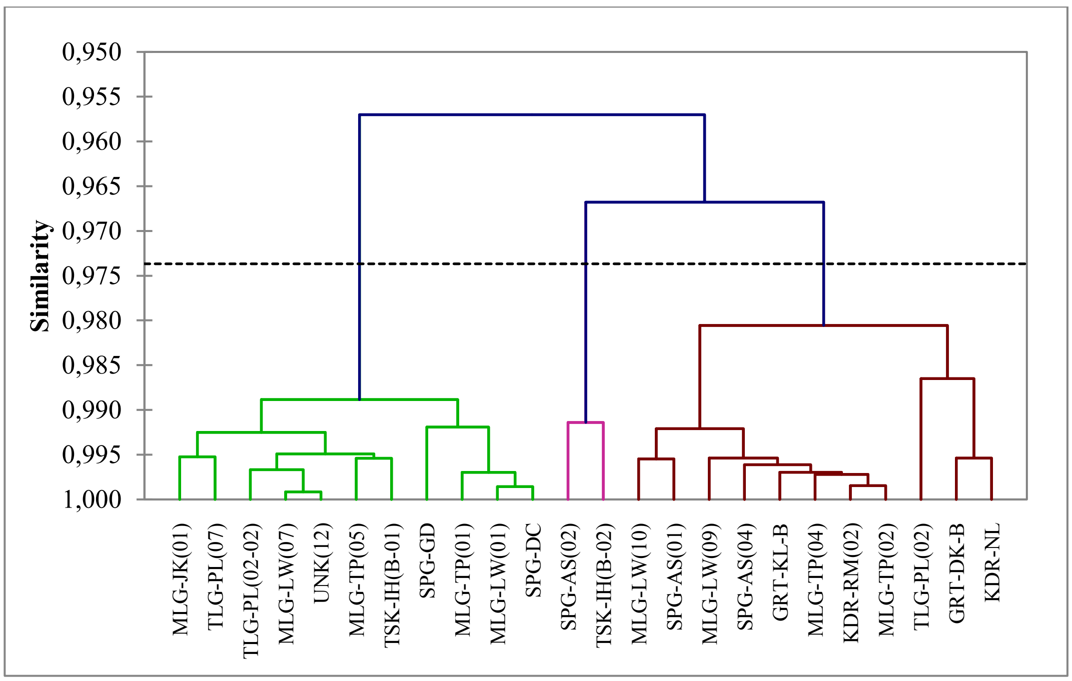

Fig. 1. Dendrogram of 24 cut leaf ground cherry genotypes based on 16 characters

Fig. 2. Characteristics variability different genotypes cut leaf ground cherry fruit derived by pure line selection from different landrace populations

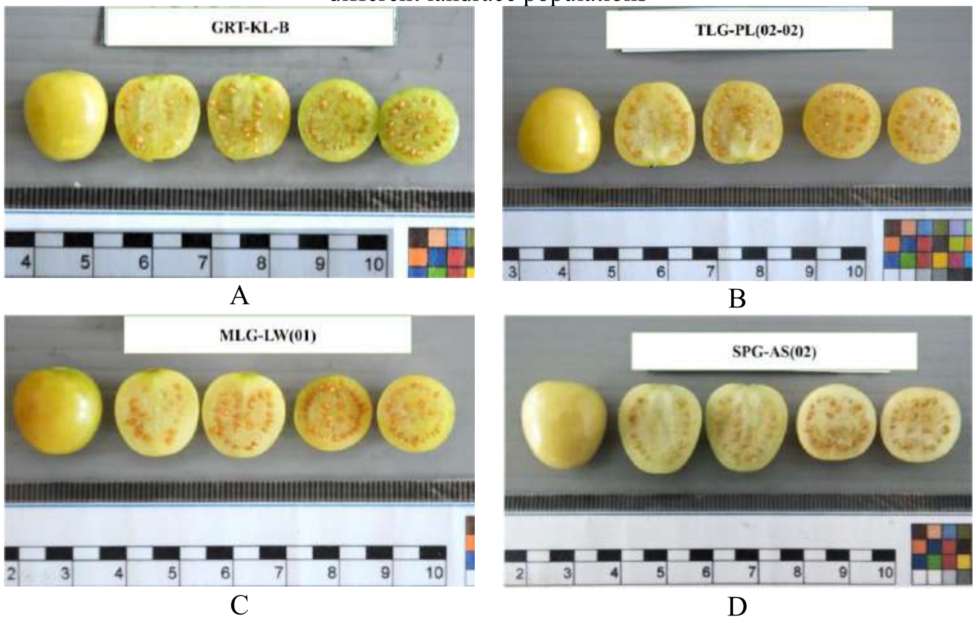

Table 4. Mean of 16 characters in 24 cut leaf ground cherry genotypes

Genotypes	Plant height at first branching (cm)	Stem diameter (cm)	Number of tertiary branches	Number of flowers per plant	Number of fruits per plant	Peduncle length (cm)	Calyx length (cm)	Calyx diameter (cm)	Fruit diameter (cm)	Fruit length (cm)	Average individual fruit weight with calyx	Average individual fruit weight without calyx	Fruit total soluble solids (TSS) (°Brix)	Fruit acidity (pH)	Fruit weight with calyx per plant (g)	Fruit weight without calyx per plant (g)
[1] GRT-DK-B	10.8	0.9	27.9	78.3	60.2	1.8	2.2	1.8	1.6	1.7	1.9	1.6	12.1	3.8	59.5	49.5
[1] GRT-KL-B	11.3	0.8	28.9	82.3	56.4	2.0	2.5	1.7	1.5	1.7	1.7	1.6	13.3	4.1	77.7	73.7
[1] KDR-NL	11.1	0.6	24.3	85.9	65.6	2.0	2.6	1.6	1.4	1.6	2.0	1.5	13.3	4.0	73.1	56.6
[1] KDR-RM (02)	8.0	1.1	33.6	110.7	78.9	1.9	2.1	1.6	1.4	1.7	1.8	1.6	12.3	3.6	106.7	91.1
[1] MLG-LW (09)	11.1	0.9	29.4	94.0	76.4	1.9	2.8	1.9	1.7	2.0	1.5	1.4	15.4	3.9	93.2	90.1
[1] MLG-LW (10)	9.1	1.4	22.0	104.2	70.5	1.8	2.5	1.6	1.4	1.5	1.8	1.7	10.3	4.1	109.5	103.3
[1] MLG-TP (02)	8.5	0.7	26.5	94.6	64.6	1.7	2.0	1.7	1.4	1.7	1.9	1.7	12.7	3.6	85.5	76.2
[1] MLG-TP (04)	12.5	0.8	30.2	82.4	62.4	1.8	1.9	1.6	1.4	1.5	1.9	1.6	12.9	3.6	82.2	68.9
[1] SPG-AS (01)	9.9	0.8	26.5	77.0	53.5	1.6	1.9	1.3	1.2	1.3	1.4	1.4	13.5	4.1	80.4	78.2
[1] SPG-AS (04)	9.2	1.0	24.8	75.0	51.1	1.8	2.1	1.9	1.6	1.8	2.5	2.0	11.9	3.4	76.9	63.7
[1] TLG-PL (02)	11.6	0.9	37.2	82.9	54.4	1.8	2.5	1.7	1.4	1.6	1.8	1.5	15.4	3.5	71.5	62.2
[2] MLG-JK (01)	13.8	1.0	26.4	127.1	118.5	1.8	2.7	1.7	1.3	1.7	2.0	1.7	13.4	3.8	156.3	135.3
[2] MLG-LW (01)	11.2	0.8	40.6	97.2	75.9	1.8	1.9	1.6	1.4	1.5	1.7	1.5	13.1	3.8	136.4	119.9
[2] MLG-LW (07)	8.4	1.1	60.5	247.9	217.8	2.0	2.7	1.6	1.4	1.6	2.0	1.9	12.0	3.8	341.3	324.5
[2] MLG-TP (01)	9.3	1.0	34.1	93.3	70.2	1.7	2.3	1.6	1.5	1.6	1.8	1.7	15.0	3.9	119.9	116.2
[2] MLG-TP (05)	10.9	0.6	23.3	87.7	78.1	1.8	2.5	1.7	1.4	1.6	1.6	1.4	13.2	4.0	118.3	108.3
[2] SPG-DC	11.0	0.7	32.6	95.3	73.0	1.9	1.9	1.6	1.4	1.5	1.5	1.3	13.3	4.0	139.5	115.1
[2] SPG-GD	8.5	0.8	23.0	82.0	55.5	1.9	2.8	1.8	1.6	1.8	1.7	1.6	11.9	4.1	122.1	117.2
[2] TLG-PL (02-02)	8.5	0.8	31.5	203.8	145.6	2.0	2.6	1.7	1.5	1.7	2.4	2.3	11.2	3.8	267.1	260.8
[2] TLG-PL (07)	8.7	1.4	49.6	160.7	131.7	1.8	2.2	1.5	1.4	1.8	2.1	1.7	11.5	3.6	202.4	170.7
[2] TSK-IH(B-01)	7.9	0.9	20.8	169.3	164.2	2.1	2.4	1.7	1.5	1.7	2.6	2.4	12.0	3.6	231.1	216.9
[2] UNK (12)	8.9	1.4	51.8	214.0	171.3	1.5	1.8	1.5	1.5	1.7	1.7	1.7	11.0	3.5	271.5	260.8
[3] SPG-AS (02)	10.6	0.8	27.5	77.1	39.4	1.8	2.3	1.4	1.3	1.5	1.6	1.5	12.3	4.0	53.9	52.2
[3] TSK-IH(B-02)	11.3	0.8	23.1	96.9	46.0	1.8	2.2	1.5	1.4	1.4	1.5	1.5	13.2	3.8	56.2	54.0
Mean	10.1	0.9	31.5	113.3	86.7	1.8	2.3	1.6	1.4	1.6	1.8	1.7	12.8	3.8	130.5	119.4
Standard error	0.3	0.0	2.1	9.9	9.5	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.3	0.0	15.8	15.3
Standard deviation	1.5	0.2	10.1	48.7	46.3	0.1	0.3	0.1	0.1	0.2	0.3	0.3	1.3	0.2	77.3	74.8

Note: number in square brackets in front of genotype indicates the group

Grouping and separate genetic resources are destined for identification of appropriate genotype with a specific trait. Genetic divergence in cut leaf ground cherry (Figure 1) revealed that 24 genotypes were grouped into three groups. Cluster 1 comprised of 11 genotypes consisting of GRT-DK-B, GRT-KL-B, KDR-NL, KDR-RM(02), MLG-LW(09), MLG-LW(10), MLG-TP(02), MLG-TP(04), SPG-AS(01), SPG-AS(04), and TLG-PL(02). Cluster 2 comprised of 11 genotypes i.e., MLG-JK(01), MLG-LW(01), MLG-LW(07), MLG-TP(01), MLG-TP(05), SPG-DC, SPG-GD, TLG-PL(02-02), TLG-PL(07), TSK-IH(B-01), and UNK(12). Cluster 3 consists of genotypes SPG-AS(02) and TSK-IH(B-02). Grouping genotypes into clusters indicate genetic divergence.

Nevertheless, this grouping of genotypes does not show the classification of specific regions.

The variability of several characteristics of cut leaf ground cherry fruit in several genotypes visually is shown in Figure 2.

Divergence through grouping genotypes shows that grouping is not based on the region of origin of genotype (Figure 1). The hope is that each genotype from the same region will form a group. This occurs because the characters observed in this study are quantitative characters whose appearance is a genotypic response to the potential for growing environments. The magnitude of environmental influences on this character can be seen from moderate and low heritability values.

Thus, although the background and genetic makeup are different, these different genotypes can respond equally or differently to the environment with the phenotype in the same quantitative metrics. This can cause grouping not to point to the origin of the region but to the similarity of character size. Although it showed no differences in genotypes from a particular region, this study had succeeded in separating the genetic potential of ciplukan from metric measurements in the form of quantitative characters measured into three groups.

Genetic divergence through multivariate techniques on ciplukan or ground cherry genotypes based on several characters makes it possible to simultaneously evaluate genotypes and allow some conclusions to be made based on the data set in Table 4. Each genotype can be directly identified the measured character so that the data can clearly be a quantitative description of the genotype. These genotypes can be selected and used directly for crop production based on the desired description criteria as new cultivar. Or, it can also be used as parental in crossing to form new populations which are then selected to produce new superior cultivar. Different groupings of genotypes provide more opportunities for selecting parental candidates and directed genetic recombination through hybridization. Genetic recombination resulting from parental hybridization measured by genetic distance through grouping or divergence will provide certainty to bring out new genotypes, perhaps even transgressive segregation will occur in certain characters so that it will be more beneficial to increase genetic capacity.

Conclusion

It was concluded that there was variability in cut leaf ground cherry characters except for fruit acidity. The coefficient of phenotypic and genetic variations shown number of tertiary branches, number of flowers per plant, number of fruits per plant, fruit weight with calyx per plant, and fruit weight without calyx per plant has high variability. High heritability's were found on plant height at first branch, number of tertiary branches, number of flowers per plant, number of fruits per plant, peduncle length, calyx length, calyx diameter, fruit length, average of fruit weight with calyx, average of fruit weight without calyx, fruit total soluble solids, fruit weight with calyx per plant, and fruit weight without calyx per plant. It can be considered as favourable attributes for cut leaf ground cherry improvement through the phenotypic selection. Dendrogram showed that 24 genotypes were grouped into three groups. Genotypes in each group show the diversity of genetic potential with a specific character and can be used as genetic material for genetic recombination to develop cut leaf ground cherry or ciplukan cultivars with high yield and quality.

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Temporal Expression of Genes *FaPYR1* and *FaCHS* in the Ripening of Strawberries *Fragaria vesca 'Californica'* and *Fragaria* x *ananassa* 'Festival'

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Abstract

Strawberries are non-climacteric fruits and highly demanded its distinctive flavour and aroma, as well as its benefits as a cancer preventive. The ripening of strawberry fruits is regulated by abscisic acid (ABA). Through the ABA signalling pathway involving receptor proteins, protein phosphatases, and protein kinases, ABA will activate the fruit ripening genes. One of the ABA receptor genes is *FaPYR*. Increased expression of *FaPYR1*, followed by the expression of fruit ripening genes such as *FaCHS*, which encodes the enzyme for the synthesis of anthocyanin, indicates that the ripening of strawberry fruits has begun. The purpose of the present study was to study the role of the expression of *FaPYR1* and *FaCHS* in the ripening of strawberry fruits. The present study analysed the level of expression of *FaPYR1* and *FaCHS* using Real-Time Polymerase ChainReaction (RT-qPCR) on two species of strawberry plants, *Fragaria* x *ananassa* 'Festival' and *Fragaria vesca* 'Californica' obtained from Banyuroto Agrotourism, Magelang, Central Java. Results showed that *FaPYR1* and *FaCHS* played a role in the ripening of strawberries but within a different duration. *FaPYR1* was expressed at a higher level in the white stage in cultivar Festival and the pink stage in Californica cultivar, while *FaCHS* was expressed higher in the red stage, both on the Californica and Festival cultivars.

Keywords: Gene expression, gene FaCHS, gene FaPYR1, Californica cultivar, Festival cultivar

Introduction

Strawberries are fruits much in demand for its distinctive flavor, color, and aroma [1]. In addition, it also contains phenolic compounds servings as antioxidants, anti-mutagenic, and anti-cancer agents capable of inhibiting cell proliferation of lung cancer, breast cancer, cervical cancer, and prostate cancer [2], [3], [4]. Strawberries are non-climacteric fruits the ripening stage of which is regulated by abscisic acid (ABA) [5], [6]. One of the events marking the ripening of strawberries is a color change to red. Anthocyanins are phenolic compounds that give red color in strawberries [7]. Anthocyanins begin to accumulate when the strawberries enter the stage of ripening, which is an implication of increased levels of abscisic acid (ABA) [8], [9].

One of intercellular ABA receptors in strawberries is FaPYR1, which is encoded by the gene *FaPYR1* [5]. Through the PYR1/PYL-PP2C-SnRK2 signaling pathway [5], [10], [11], ABA activates the fruit ripening genes [12], one of which being *FaCHS* [13]. Synthesis of anthocyanins in strawberries is regulated by the gene *FaCHS* (*Fragaria Chalcone Synthase*). This gene encodes the enzyme chalcone synthase, which then catalyzes the synthesis of anthocyanins. *FaCHS* and other ripening genes are activated by abscisic acid (ABA). The mechanism is that when the ABA hormone along with metabolites, enzymes, hormones, and transcription factors work together to induce *FaCHS* in strawberries [14], [15], [16], then *FaCHS* catalyzes the formation of naringenin compound, which is a precursor of some flavonoid-derived compounds of malonyl-CoA and *p*-coumaroyl-CoA [7]. Furthermore, naringenin stimulates the formation of new genes, such as CHI (Chalcone Isomerase), which can activate anthocyanins that give the color phenotype to the strawberry fruits [7].

The purpose of the present study was to investigate the expression of *FaPYR1* and *FaCHS* and their role in the ripening of strawberries *Fragaria vesca* 'Californica' and *Fragaria ananassa* 'Festival'.

Materials and Methods

Materials

The materials used in this study were strawberries of Californica cultivars (*Fragaria vesca* subs. *californica* 'Californica') and Festival *(Fragaria* x *ananassa* 'Festival'). The primers used were based on the study of [5], namely:

Primers of gene *FaPYR1*:

- *FaPYR1*-F5'-ATGGAGAAACCATCATCGGC-3'
- *FaPYR1*-R5'-TCAGACCTGGGGAGTTAGCG-3'

Primers of gene *Actin*:

- *Actin* 5' TGGGTTTGCTGGAGATGAT 3'
- *Actin* 5' CAGTAGGAGAACTGGGTGC 3'

Primers used for temporal expression of gene *FaCHS* referred to those of [17]:

- *FaCHS* – F5'-GCCTTTGGTGTTGCTTGA CT-3'
- *FaCHS* – R5'-CCCAGGAACTTTATCGAG GA-3'
- 26S-18S RNA *Housekeeping gene* – 5'-ACCGATGTTAATCACTGGTCATCGTCG-3'

Morphological Observation of Growth and Development of Strawberries

Strawberries of Californica and Festival cultivars were picked in the green stage (8 days), white (18 days), pink (22 days), and red (25 days) with 3 replications and then measured for vertical and horizontal diameter.

Total RNA Isolation

RNA isolation was based on the method of [18] with a modification of the 3% $CTAB_3$-LiCl method that could be used to isolate the RNA of strawberries. The isolates were then visualized by gel electrophoresis at a concentration of 0.8%.

cDNA Synthesis

cDNA synthesis was conducted by means of the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit.

The composition of the RT-PCR buffer was 2µL of oligo dT primer, 2µL of 5x RT buffer, 4µL of dNTP mix, 1µL of RNase inhibitor, 1µL of reverse transcriptase, and 9µL of distilled water.

Furthermore, incubation was performed at 42 °C for 1 hour and at 70 °C for 5 minutes.

DNA Amplification

DNA was amplified by Polymerase Chain Reaction (PCR) method. The composition of the PCR buffer was 12.5µL of KAPA Extra HotStart, forward primer, 1µL of reverse primer and 5.5µL of distilled water. Furthermore, it was amplified by a PCR machine and set in accordance with the procedures in the protocol of KAPA Taq Extra Hotstart Readymix PCR Kit, consisting of pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 40 s with a repetition of 35 cycles, and then post-elongation at 72 °C for 3 min for *FaPYR1*; *FaCHS* was amplified using the same protocol but with *annealing* at 54 °C.

Real-Time Polymerase Chain Reaction

The level of *FaPYR1* expression was quantified using a Real-Time Polymerase Chain Reaction (RT-qPCR). RT-qPCR was based on the protocol of KAPA SYBR®FAST qPCR Master Mix (2x) Universal Kit. The composition of RT-qPCR buffer was 7.2µL of PCR grade water, 10µL of master mix kit, 0.4µL of forward primer, and 0.4µL of reverse primer.

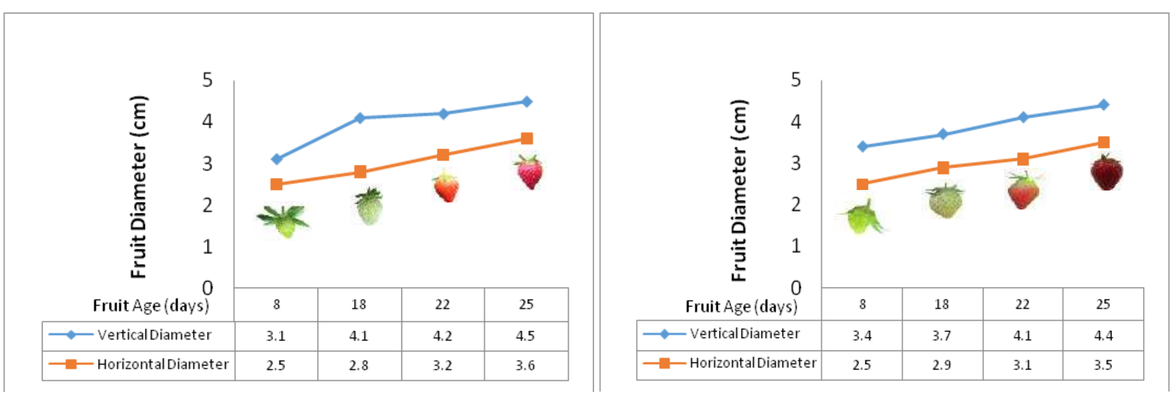
The steps of RT-qPCR were performed using the optimization in accordance with the protocol of KAPA SYBR®FAST qPCR Master Mix (2x) Universal Kit. The process of RT-qPCR was performed in 40 cycles at a temperature below 95 °C for 3 min, 95 °C for 3 s, 60 °C for 20 s, 65 °C for 5 s, followed by 60 cycles increase from 65 °C to 95 °C with an addition of 0.5 °C per cycle for 5 s.

Data Analysis

The level of gene expression was analyzed using a relative quantization based on the method of [19] or the compare C_q method/$\Delta\Delta C_q$ using *Actin* as the reference gene.

Results and Discussion

Based on fruit morphology, Californica and Festival cultivars entered the ripening stage at day 22 and ripened at day 25 (Figure 1). In the early stages of ripening, the fruits were pink with some of the receptacles were still white and the achenes were beige. In the final stage of ripening, the receptacles were red, whereas the achenes were brown although some were also red.



(a) (b)

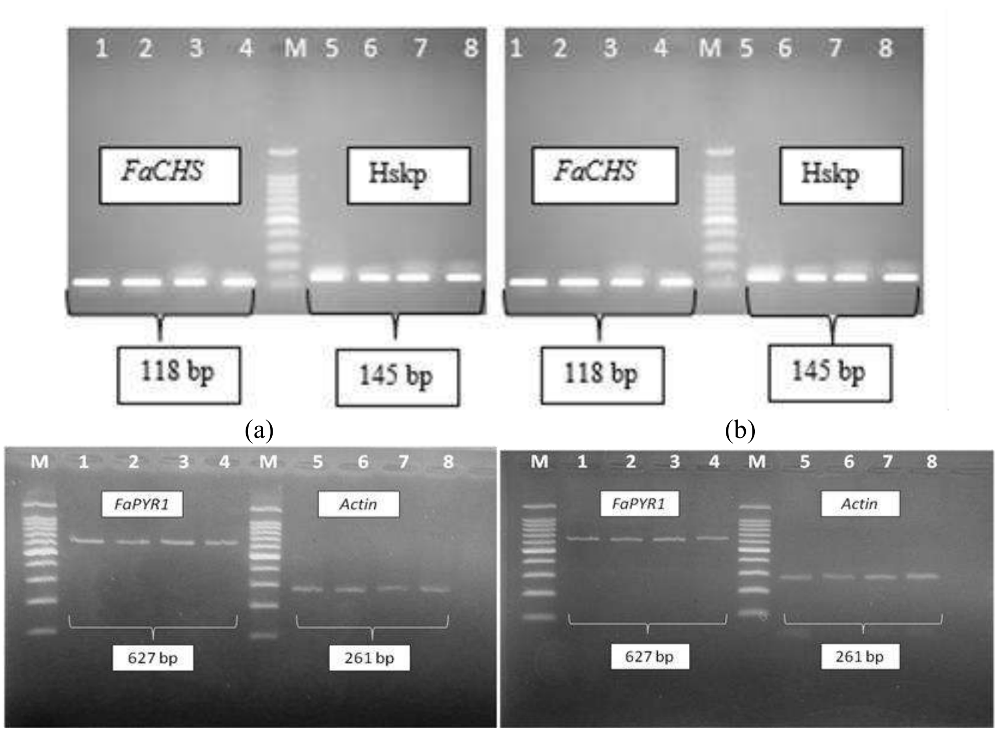
Fig. 1. Growth and development of strawberry fruit. (a) Californica cultivar; (b) Festival cultivar

Total RNAs of strawberries were isolated and the isolates could be used for the next stage. The isolates of total RNAs were of good quality since it was not smeared. RNAs obtained from Californica cultivar measured 200-1000 bp, while those of Festival cultivar were 200-750 bp.

Based on the study of [18], RNAs of strawberries (*F.* x *ananassa* D.) has a molecular weight of 200-2000 bp. Thus, the total RNA bands obtained by this study were total RNA bands. The types of RNA obtained with the size were mRNA, hnRNA, and cytoplasmic rRNA. Genomic DNA contamination was also not detected by electrophoresis. Thin bands indicate low concentrations of total RNA. The concentrations of total RNA showed an A260/A280 ratio of 2.0±0.1. However, A260/A230 ratio showed values below 2.0, indicating polysaccharide contamination or residual reagents [20]. This was because strawberries contain many polysaccharides and polyphenols. Nonetheless, the total RNA obtained could be used for further analysis.

The total RNA was then used as the template for reverse transcription polymerase chain reaction (RT-PCR) for cDNA synthesis. The product was subsequently used as the template for Polymerase Chain Reaction (PCR) to test whether or not the target genes *FaPYR1* and *FaCHS* with the control genes *Actin* and 26S-18S RNA Housekeeping gene were successfully amplified.

The product of the PCR is shown in Figure 2.



(c) (d)

Fig. 2. Electropherogram of PCR products

(a) *FaCHS* and housekeeping gene of Festival cultivar;
(b) *FaCHS* and housekeeping gene of Californica cultivar;
(c) *FaPYR1* and *Actin* of Californica cultivar; and
(d) FaPYR1 and Actin of Festival cultivar.

***Notes**: M, Marker,1&5=green phase; 2&6=white phase; 3&7=pink phase; 4&8=red phase.*

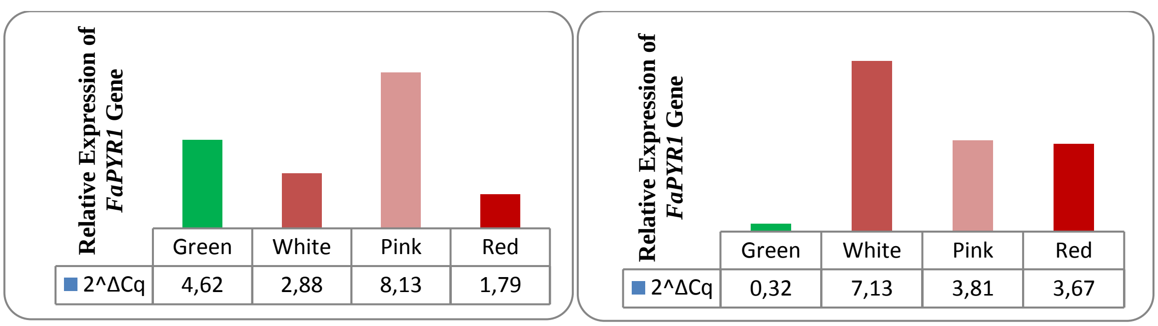
The target genes *FaPYR1* and *FaCHS* were successfully amplified at green, white, pink, and red stages both for Californica and Festival cultivars (Figure 2). *FaPYR1* measured 627 bp, while *FaCHS* was 118 bp. The control genes *Actin* and 26S-18S RNA Housekeeping gene were also successfully amplified in the respective stages of both cultivars with a length of 261 bp and 145 bp, respectively. These results indicate that *FaPYR1* and *FaCHS* were detected at each stage of growth and development in both cultivars of strawberries. Detection of *FaPYR1* and *FaCHS* showed that the activity of those genes was associated with the role of ABA in the growth and development, especially at the ripening stage of strawberries.

The levels of *FaPYR1* and *FaCHS* expression analyzed using Real-Time Polymerase Chain Reaction (RT-qPCR) showed the extent of the relative expression of *FaPYR1* and *FaCHS* during growth and development of strawberries. The relative expression of *FaPYR1* and *FaCHS* at each stage of growth and development of strawberries was based on the Cq (quantitative cycle) value of FaPYR1 normalized to the Cq value of Actin and that of FaCHS was normalized to the Cq value of 26S-18S RNA Housekeeping gene. The Cq value is the cycle number when the fluorescent signal passes through the threshold. A threshold is the level of the fluorescence signal that reflects a statistically significant increase compared to the signal at a predetermined baseline.

A Cq value is inversely proportional to the relative level of gene expression. A low Cq value, or reach the threshold earlier, shows a higher level of relative expression [21].

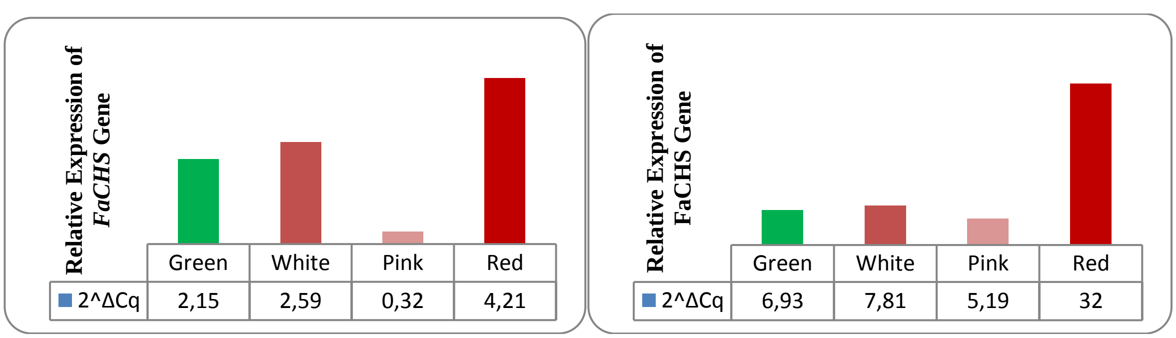
As shown by the study of [5], the relative expression of *FaPYR1* (Figure 3) at the pink stage is higher than that in the red stage, both on the Californica cultivar and Festival cultivar. Results obtained for the relative expression of *FaCHS* also indicated conformity with those of [17], in which *FaCHS* was expressed at the highest level at the red stage (Figure 4). These results indicated that both genes played a major role in the ripening of strawberries, especially on the onset of ripening.

Abscisic acid (ABA) is the regulator of the ripening of non-climacteric fruits, including strawberries. It would activate the ripening genes, such as sugar formation genes, discoloration genes, flavor and aroma formation genes, as well as the fruit softening genes [12], [22]. Such ripening genes are usually activated through the PYR1-PP2C-SnRK2 pathway [5], [23].



(a) (b)

Fig. 3. Relative expression levels of *FaPYR1* in (a) Californica cultivar and (b) Festival cultivar



(a) (b)

Fig. 4. Relative expression levels of *FaCHS* in (a) Californica cultivar and (b) Festival cultivar

In strawberries, FaPYR1 is an ABA receptor which, when it has formed a complex with ABA, would bind to PP2C and activate SnRK2 to induce the expression of the ripening gene, *FaCHS* [16]. In Californica cultivar, the relative expression of *FaPYR1* at the pink stage increased from the previous stage. This suggests that ABA signaling for the ripening process has begun. Along with the elevated levels of ABA in the pink stage, activation of ripening genes began, as shown by the receptacle color that started to turn pink, indicating that *FaCHS* has been activated.

However, the expression level of *FaCHS* remained low since it was still in its early stages of expression and there were not many precursors available for the formation of anthocyanin. The precursor of anthocyanins is aglycone (anthocyanidins) which is esterified with one or more groups of sugar (glycone). The sugar groups on anthocyanin were varied, but mostly in the form of glucose, rhamnose, galactose, and arabinose. When the precursors are available in very small amounts, *FaCHS* expression remains low [7].

Entering the red stage, the levels of *FaPYR1* expression decreased, despite the increase in ABA.

This is because the increasingly higher levels of ABA will increase levels of PP2C (protein phosphatase 2C), leading to an increasingly greater ratio of PP2C to ABA receptors. The increasing ratio will decrease plants' sensitivity to ABA so that when the plant is not sensitive to ABA, leading to decreased expression of receptor genes, such as *FaPYR1* will [24]. However, the increased expression of *FaCHS* in the red stage could be due to adequate availability of precursors for the formation of anthocyanin at this stage. It can also be assumed that *FaPYR1* and *FaCHS* were not expressed at the same time so as to have different expression levels. *FaPYR1* plays a role early as a receptor for ABA, while *FaCHS* must be activated by a transcription factor whose activity is induced by SnRK2. SnRK2 proteins cannot be activated without FaPYR1-ABA complex. Therefore, *FaCHS* expression occurs after *FaPYR1* expression and is limited by the availability of precursor which is a derivative of sugar compounds. Synthesis of sugar compounds is catalyzed by sugar-forming genes which are also activated by ABA. Since anthocyanins enter the end pathway of metabolism, it can be ascertained that *FaCHS* expression is also higher in the red stage, which is the final stage of strawberry ripening.

Similar results were also obtained for Festival cultivar, in which *FaPYR1* expression at the pink stage was higher than that of the red stage with *FaCHS* expression in the red stage higher than that of the pink stage. The highest level of *FaPYR1* expression in Festival cultivar occurred in the white stage, which could be due to environmental stresses that caused an increased relative expression of *FaPYR1* in order to play a role in ABA signaling mechanism to provide a defense response.

The fluctuating result of *FaPYR1* expression during fruit development and ripening is quite common in strawberry [25].

Results showed that *FaPYR1* and *FaCHS* played a role in the ripening stage of strawberries, especially in the formation of the fruit color through anthocyanin synthesis. The higher level of anthocyanins in the red stage indicated that in this stage *FaCHS* expression was also higher than the previous stage. However, since *FaPYR1* played a role earlier as an ABA receptor to activate *FaCHS*, expression of the gene at the pink stage was higher than that in the red stage. Similar results were obtained for both Californica and Festival cultivars.

Conclusion

FaPYR1 and *FaCHS* are genes involved in the ripening of strawberries Californica and Festival cultivars. *FaPYR1* encodes ABA receptor proteins, whereas *FaCHS* encodes enzymes for the synthesis of anthocyanins. *FaPYR1* was expressed highest in the white stage possibly as a defense system and during the ripening stage expressed higher in the pink stage while *FaCHS* was expressed highest in the red stage in both Californica and Festival cultivars.

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Why is Calcium, Can Increase the Amount of Mangosteen Fruit (*Garcinia mangostana* L.) Quality?

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Abstract

Low effectiveness of Ca absorption in mangosteen fruit makes high levels of yellow sap contamination. This study aimed to: (1) determined the effect of Ca in yellow sap contamination on mangosteen fruit; (2) know mechanism of absorption and distribution of Ca in mangosteen plants. The study was carried out in the mangosteen forest in Cengal, Bogor, Indonesia. The experiment was carried out using Factorial Randomized Block Design with 3 replications, consisting of the treatment of Ca and B fertilizer doses as the first factor, consisting of 6 levels, namely: (1) Control (without Ca and B), (2) 2.8 g B/tree/year, (3) 3.2 kg of Ca dolomite/tree/year, (4) 3.2 kg of Ca calcite/tree/year calcium, (5) 3.2 kg of Ca dolomite/tree/year+2.8 g B/tree/year, and (6) 3.2 kg of Ca calcite/tree/year+2.8 g B/tree/year. The second factor consisted of 2 levels, namely: (1) Ca and B application at the time of the antesis and 1 week after the antesis. (2) Ca and B application at the time of anthesis and at the end of stage I. The results showed: (1) Ca application was able to reduce the contamination of yellow sap on the mangosteen aryl reached 53% and on the rind reached 46% and improved the quality of fruit, without Ca produced yellow sap contamination 91.66% on aryl and 86% on the rind; (2) Ca was absorbed and distributed by the plant through several mechanisms, such as through xylem in root and continue to the stem and then to pedicel before absorbed into the fruits.

Keywords: xylem, cell wall, pectin, fruits, yellow sap

Introduction

The main problem in the production of mangosteen fruit in Indonesia is the presence of yellow sap contamination in aryl and mangosteen rind. The presence of yellow sap contamination on the mangosteen fruit will affect the quality of the fruit both the appearance and taste of the mangosteen fruit. Calcium is a nutrient that is not mobile in plant tissues. Calcium translocation from the root to the canopy of the plant is influenced by transpiration, therefore one of the obstacles that arises is the low effectiveness of calcium absorption into the mangosteen fruit tissue. Most of the calcium absorbed from the roots will be directly localized to the leaf tissue, because the nature of calcium which is not mobile in xylem and its translocation in plant tissues is affected by the process of plant transpiration (Drazeta *et al*., 2004; Sergio *et al*., 2011). Leaves as active plant tissues transpire will attract calcium from the roots and become competitors for the fruit (Pessarakli 2002; Cassandro *et al*., 2013). Therefore, an effort is needed to improve the translocation of calcium to the fruit (Huang *et al*., 2005).

Calcium application by spraying directly on the fruit surface can increase the calcium content of the pericarp of the fruit (Clark *et al*., 1987; Rosen *et al*., 2006; Dorly 2009; Song Wen-Pei *et al*., 2014), but this is not practice and less efficient on mangosteen in terms of its application in the field. Calcium application in wrong times through the soil will increase the calcium content in the leaf tissue, but does not increase calcium in the mangosteen rind (Dorly 2009; Depari 2011).

This was clarified in the research conducted by Purnama (2014) who obtained results that there was an increasing in calcium content in the pericarp of the fruit along with an increasing in the calcium fertilizer dose given.

Other studies indicate the relationship between boron content and increased absorption and function of calcium in reducing the contamination of yellow sap on mangosteen fruit (Murat *et al*., 2009). Saribu (2011) showed that the application of calcium together with boron through the soil decreased the yellow sap contamination in aryl up to 0%. Similar results are shown in research conducted by Pechkeo *et al*., (2007) and Kurniadinata (2016b) that the application of calcium and boron to the mangosteen fruit can increase the calcium content of the pericarp and reduce the potential for yellow sap contamination in the mangosteen fruit. However, it is not yet clear whether the link between boron in supporting calcium uptake and translocation to the mangosteen fruit tissue and its influence on different sources of calcium on the decrease of yellow sap contamination in pericarp and aryl mangosteen. Therefore, it is necessary to obtain calcium absorption and translocation mechanisms and the most effective and efficient application of calcium and boron techniques, to increase calcium absorption in fruit tissue and reduce the contamination of yellow sap on mangosteen fruit. This study aimed to: (1) determine the effect of calcium and calcium sources in overcoming yellow sap contamination on mangosteen fruit; (2) know the mechanism of absorption and translocation of calcium in mangosteen.

Materials and Methods

Place and time

The study was conducted in the mangosteen garden in the Manggis Karya Mekar Farmer Group, in Cengal Village, Karacak Village, Leuwiliang, Bogor, Indonesia. The research was located at an altitude of 390-398 m above sea level (ASL). Leuwiliang mangosteen garden is dominated by productive mangosteen plants that are more than 20 years old. This garden is located at an altitude of 390-398 m ASL, with 6-30% wavy and sloping topography, high podzolic soil type with high clay texture and pH ranging from 4.30-5.50. Soil chemical analysis and plant tissue were carried out at the Laboratory of the Soil Research Institute, Bogor and the quality of fruit at the Post Harvest Laboratory of the Faculty of Agriculture, Bogor Agricultural University.

Material

The plant material used was mangosteen plants aged approximately 20 years and has been producing. The selection of sample plants was based on good and relatively uniform plant growth conditions. The level of uniformity was assessed based on the condition of the trees in the garden, which was based on similarity in stem diameter, crown size, plant height and suitability of maintenance history, with a view to reducing the diversity of plant conditions.

Research methods

The experiment was carried out using factorial randomized block design (RBD) with 3 replications, consisting of the treatment of calcium and boron fertilizer doses as the first factor, consisting of 6 levels, namely:

1) Control (without calcium and without B)
2) 2.8 g B/tree/year (6.09 g borate 46/tree/year)
3) 3.2 kg of calcium dolomite/tree/year (10.67 kg dolomite/tree/year)
4) 3.2 kg of calcium calcite/tree/year (7.11 kg of calcite/tree/year)
5) 3.2 kg of calcium dolomite/tree/year + 2.8 g B/tree/year
6) 3.2 kg of calcium calcite/tree/year + 2.8 g B/tree/year

While the second factor was the number of stages of Calcium application and boron in mangosteen per year (T), which consisted of 2 levels, namely:

1) Calcium and boron were applied at the time of the antesis and at the start of the first stage (1 week after the antesis (WAA) half of the dose at specified each time.
2) Calcium and boron were applied at the time of anthesis and at the end of stage I (4 WAA) half of the dose at specified each time.

Each treatment level consists of one plant so that 36 mangosteen plants were needed (approximately 20 years of age and have fruited) that are relatively uniform in the experimental location. The calcium sourced from Dolomite ($CaMg(CO_3)_2$) and Calcite ($CaCO_3$), while boron sourced from Borat 46 fertilizer.

Research Result

Application of Calcium and Boron

Application of calcium and boron was able to reduce the percentage of fruit contaminated with yellow sap on aryl, yellow sap contamination scores on aryl, and the percentage of polluted fruit segment, both in the first year and in the second year of the experiment (Table 1). In the first year, the application of dolomite + boron was able to reduce the percentage of fruit contaminated with yellow sap on aryl to be 53%, much lower compared with no calcium and boron (control) which produced yellow sap contamination in aryl by 91.66%. Lower contamination scores were also found in dolomite + boron treatment of 1.68, while control showed a contamination score of 3.01.

In the second year, the application of dolomite + boron reduced the percentage of fruit contaminated with yellow sap on aril to 31.66%, which was not significantly different from the application of dolomite (36.33%) and calcite + boron (33.00%), but significantly different from the control (62.66%). Whereas the percentage of contamination per fruit, dolomite + boron application was significantly different from the control but not significantly different from other applications, both in the first and second year.

Table 1. Fertilization of calcium and boron in mangosteen, on the percentage of fruits contaminated with yellow sap on aryl tree^{-1}, percentage of polluted segment and yellow sap contamination scores on aril for two years

Application of calcium and boron	Yellow sap contamination on aril					
	Contaminated fruit.tree^{-1} (%)		Contaminated fruit segment (%)		Score (1-5)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Control	91.66 a	62.66a	30.4 a	33.3 a	2.96a	3.01a
Boron	85.00 a	57.66a	26.5 ab	26.7 b	2.45b	2.45b
Dolomite	63.33 b	36.33c	19.3 c	18.3 c	1.81d	1.88c
Calcite	66.66 b	42.33b	24.2 bc	20.9 c	1.95cd	1.80cd
Dolomite + boron	53.33 c	31.66c	18.7 c	17.3 c	1.80d	1.68d
Calcite + boron	68.33 b	33.00c	22.2 bc	21.8 c	2.05c	1.81c

Note: *Scoring data were tested using the Kruskal Wallis rank test. The contamination score is based on a score of 1-5 with a value of 1 (best/without contamination) up to a score of 5 (worst/has the highest contamination score). The numbers followed by the same letter in the yellow sap score column showed no difference significantly based on Dunn's test of 5%, in the column % of contaminated fruit per tree and % of contaminated fruit segment per fruit showed no significant difference based on 5% DMRT test.*

The application of calcium and boron reduce the percentage of fruit contaminated with yellow sap on the rind and the yellow sap contamination score on the rind during the two-year trial (Table 2). The application of dolomite + boron reduced the percentage of yellow sap contamination on the rind to 76.66% in the first year, while the control produced 88.33%. In the second year of the experiment, application of dolomite + boron decreased the percentage of yellow sap contamination on the rind to 46.33%, while the control was still above 85%. Likewise, in the yellow sap contamination score on the rind, the application of dolomite + boron showed lower sap contamination scores compared to the control, and was not significantly different from other applications of calcium and boron.

Table 2. Fertilization of calcium and boron in mangosteen, on the percentage of fruit contaminated with yellow sap on the rind. tree^{-1} and yellow sap contamination score on fruit rind for two years

Application of calcium and boron	Yellow sap contamination on Rind			
	Contaminated fruit. tree^{-1} (%)		Score (1-5)	
	Year 1	Year 2	Year 1	Year 2
Control	88.33a	86.00a	3.01a	3.00a
Boron	86.66ab	73.00b	2.73b	2.58b
Dolomite	83.33ab	52.00cd	2.26c	2.13c
Calcite	85.00ab	57.00c	2.20c	2.18c
Dolomite + boron	76.66bc	46.33d	2.11c	2.08c
Calcite + boron	71.66c	50.00d	2.11c	2.15c

Note: *Scoring data were tested using the Kruskal Wallis rank test. The contamination score is based on a score of 1-5 with a value of 1 (best/without contamination) up to a score of 5 (worst/has the highest contamination score). The numbers followed by the same letter in the yellow sap score column showed no difference significantly based on Dunn's test of 5%, in the column % of contaminated fruit per tree and % of contaminated fruit segment per fruit showed no significant difference based on 5% DMRT test.*

Application Time of Calcium and Boron

When the application of calcium and boron had an effect on the percentage of fruit contaminated with yellow sap on aril in the second year of the experiment, it did not give an effect on the decrease in the yellow sap contamination score in aril in the same year. The application of calcium and boron only affects the yellow sap contamination score in the first year of application.

Whereas in the percentage of contaminated fruit segment, calcium and boron application time did not give effect both in the first year and the second year of the experiment (Table 3).

Table 3. Time for fertilizing calcium and boron in mangosteen, on the percentage of fruit contaminated with yellow sap on aryl tree^{-1}, yellow sap contamination score on aryl and the percentage of polluted fruit segment for two years

Application of calcium and boron	Yellow sap contamination on aril					
	Contaminated fruit.tree^{-1} (%)		Contaminated fruit segment (%)		Score (1-5)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Anthesis + 1 WAA	70.00	42.33 b	0.23	0.22	2.08b	2.10
Anthesis + 4 WAA	72.77	45.55 a	0.23	0.23	2.25a	2.11

Note: *Scoring data were tested using the Kruskal Wallis rank test. The contamination score is based on a score of 1-5 with a value of 1 (best / without contamination) up to a score of 5 (worst / has the highest contamination score). The numbers followed by the same letter in the yellow sap score column showed no difference significantly based on Dunn's test of 5%, in the column % of contaminated fruit per tree and % of contaminated fruit segment per fruit showed no significant difference based on 5% DMRT test.*

The application of calcium and boron did not effect on the decreasing in the percentage of yellow sap contamination on the rind both in the first and second year. However, the application time of calcium and boron has an effect on the yellow sap contamination score on the rind in the first year of application, whereas in the second year it does not give effect. Application of calcium and boron at the time of antesis + 4 WAA gave the worst yellow sap contamination score compared to the application at the antesis + 1 WAA (Table 4).

Table 4. Time for fertilizing calcium and boron in mangosteen, on the percentage of fruit contaminated with yellow sap on the rind $tree^{-1}$ and yellow sap contamination score on fruit rind for two years

Application of calcium and boron	Yellow sap contamination on Rind				
	Contaminated fruit $tree^{-1}$ (%)		Score (1-5)		
	Year 1	Year 2	Year 1		Year 2
Anthesis + 1 WAA	82.22	60.77	2.35	b	2.37
Anthesis + 4 WAA	81.66	60.66	2.46	a	2.33

Note: *Scoring data were tested using the Kruskal Wallis rank test. The contamination score is based on a score of 1-5 with a value of 1 (best/without contamination) up to a score of 5 (worst/has the highest contamination score). The numbers followed by the same letter in the yellow sap score column showed no difference significantly based on Dunn's test of 5%, in the column % of contaminated fruit per tree and % of contaminated fruit segment per fruit showed no significant difference based on 5% DMRT test.*

In the second year, the application of calcium and boron at the time of antesis + 1 WAA was able to reduce the percentage of fruit contaminated with yellow sap on fruit aryl better than the application of calcium and boron at the time of antesis + 4 WAA. It is suspected that calcium and boron applications can meet calcium and boron requirements after two years of application to support fruit growth and development at a later stage. Whereas in the first year, the application time can only reduce the yellow pollutant sap score on aryl and fruit rinds, with the application when antesis + 1 WAA decreases the yellow sap contamination score lower than the application time at antesis + 4 WAA.

Discussion

The quality of mangosteen fruit is related to the presence of yellow sap on the rind and fruit aryl (Kurniadinata *et al*., 2016a). The results of this study indicate a decrease in the percentage of fruit contaminated with yellow sap and yellow sap contamination scores on aryl and rind, since the first year of the experiment. This has become an indicator that calcium is the main important element in the decline. The combination of calcium application (both from dolomite or calcite) and boron reduce the percentage of polluted fruit on aryl and rind as well as yellow sap contamination scores on aryl and rind, both in the first year and in the second year of the experiment. It is suspected that with the application of calcium, there is an increase in uptake and translocations of calcium into the fruit tissue. In addition to calcium, a combination of calcium and boron will guarantee the supply of boron to fruit growth and development. Boron has the same function in increasing cell wall strength as well as calcium (Hu *et al*., 1996). Therefore, boron is thought to support the function of calcium in increasing the strength of the wall of the yellow epithelial cell channel epithelial cells.

The combination of calcium and boron will increase the resistance of the cell wall channel to the risk of rupture when there is pressure on the channel. Limpun-Udom (2001) in his study found that calcium and boron content in the normal mangosteen rind which was not contaminated with yellow sap was higher than the fruit that experienced yellow sap contamination. Lim *et al*., (2001) added that boron has an important function in supporting calcium function in plant tissues especially as one of the constituent components of cell walls. The presence of boron elements will support increased resistance and rigidity of the yellow sap channel cell wall. Marschner (1995) and O'Neill *et al*., (2004) that just like calcium, boron functions as a constituent of cell walls, functioning to improve the stability and firmness of cell wall structures and improve plasma membrane integrity. Furthermore Kobayashi *et al*., (1996) on the results of their study reported that boron as boric acid bound together with two rhamnogaladuronan II (RG II) chains forms a boron-polysaccharide complex. These two Rhamnogaladuronan II molecules are linked to each other by boric acid to form a boron-polysaccharide complex. Application of calcium both sourced from dolomite and calcite with boron will ensure the availability of calcium and boron during fruit growth and development and support the formation and development of cell walls in the phase of fruit growth and development in the generative phase of the plant. The presence of pressure on the fruit endocarp due to the rapid growth of seeds and aryl cause the yellow sap channel to be susceptible to damage. The presence of calcium from both dolomite and calcite and boron will increase the cell wall strength of the yellow sap canal so it is not easily broken. Both dolomite and calcite have a relatively similar effect in reducing the score of yellow sap contamination both on aryl and mangosteen rind. This shows that the two sources of calcium can be used to overcome the contamination of yellow sap on the mangosteen fruit. However, Boron's ability to reduce the percentage of fruit contaminated with yellow sap and contamination scores has not been as large as the decrease obtained with calcium application.

Calcium requirements for plants occur when entering the generative stage, especially during anthesis (Hu *et al*., 1996; Pechkeo *et al*., 2007; Nicola *et al*., 2001). In this stage, fruit plants will absorb all the nutrients needed for the growth and development of flowers and fruit (Ropiah 2009).

The nature of the toxic boron element causes boron cannot be applied excessively. Marshner (1995) states that boron is toxic to plants at different levels, this difference is related to the function of boron and the need for boron. Boron is mainly absorbed maximally during cell wall synthesis, especially at the time of lignin formation and as a constituent of cell walls. For mangosteen plants, Martias (2012) in his study found that optimum leaf boron content (86.5 ppm) eliminated yellow sap contamination to a minimum (2.86%), but increased boron leaves up to 130 ppm caused yellow sap contamination to increase by 40.7%. From the results of this study it can be seen that the application of boron can reduce yellow sap contamination but in certain amounts boron can act as toxic for plants.

Several studies have shown that calcium can increase the tolerance level of plants to the toxic properties of boron (Simon *et al*., 2011). As stated by Tisdale *et al*., (1985) in his research that there is a relationship between calcium and boron on plants. When calcium is in sufficient quantities, plants will become more tolerant of the presence of boron. The presence of calcium is known to be able to increase the tolerance level of plants against boron poisoning by regulating boron transport in plant cells, and protecting cells from the entry of boron into plant cells in excess amounts. This will improve the efficiency of the function of boron as one of the constituents of the cell wall and is able to improve the quality of fruit, and this function is closely related to the presence of calcium.

There is a relationship between fruit development with calcium requirements available in the soil, namely the increase in calcium absorption into the fruit tissue during fruit development. The increase occurs following the fruit development stage, mainly occurs in the early stages of fruit development and then decreases as the level of fruit maturity increases. Mentioned by Tomala *et al*., (1989) that calcium absorption into fruit tissues occurs continuously and fluctuates in the process of fruit development. This statement is in accordance with Faust (1989), Wilkinson and

Perring (1961), Ford and Quinlan (1979), Fuhr and Wieneke (1974), Hu *et al*., (1996), Pechkeo *et al*., (2007) and Wilsdorf (2011) which states that in general the absorption of calcium by fruit occurs during the initial stages of fruit growth and development. Rapid absorption of calcium and boron by plants mainly occurs at the beginning of fruit growth and development, which is translocated via xylem to the fruit.

Increasing the size of the mangosteen fruit causes the fruit to be susceptible to yellow sap contamination in aryl and fruit rinds due to pressure due to fruit development. The pressure occurs due to the difference in the growth rate between aryl and seeds to the rind of the fruit. This insistence has the potential to cause rupture of the yellow sap channel in the pericarp tissue of the fruit which then pollutes the aryl (Poerwanto *et al*., 2010).

The application of calcium and boron through rooting at the time of anthesis can meet calcium requirements, especially in the fast stadia of fruit development, i.e., at 1-4 WAA. Stage 1 of fruit growth becomes critical time for Ca up to support fruit growth and development. Poovarodom (2009) explained that the mangosteen fruit consists of three fruit development stages namely I 1-4 WAA stage, stage II 5-13 WAA, and stage III 14-15 WAA. Therefore, the application of calcium and boron which is done twice during antesis + 1 WAA is thought to be able to increase absorption and translocation of calcium to fruit tissue through xylem compared to the application at antesis + 4 WAA (Figure 1).

Application of calcium and boron at the time of antesis + 4 WAA is thought to be less effective in increasing uptake and translocation of calcium and boron into the fruit tissue. This is because when 4 WAA the need for calcium and boron for the growth and development of fruit is not as high as the requirement at the antesis + 1 WAA. Explained by Rigney and Wills (1981) and Poovarodom (2009) that during the development of the mangosteen fruit, calcium needs in the cell wall will increase but will then decrease before fruit ripening.

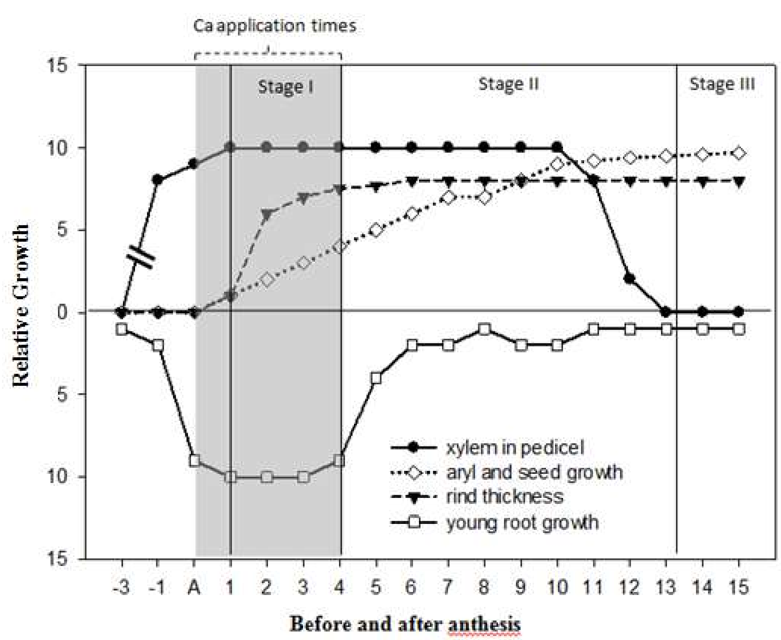

Fig. 1. Relationship model of calcium absorbed and translocation based on young root growth, fruit growth, and xylem condition in pedicel. X axis ([-] = Weeks before anthesis; [A] = anthesis; [+] = weeks after anthesis). Y axis (xylem, aryl and seed, rin, and young root growth (not to scale). (source: Kurniadinata *et al*., 2017)

The category of fast fruit growth in mangosteen occurs at 1-4 WAA. At this time the elements of calcium and boron will be translocated to fruit tissue in large quantities because the fruit becomes a strong sink against various nutrients (Marschner 1995). In this stage the flow of calcium and boron translocation that was previously dominant towards the leaves will move towards the fruit. Application of calcium and boron at the time of antesis + 4 WAA will be less effective than the application of calcium and boron during antesis + 1 WAA because during the second application at 4 MSA, the need for calcium and boron does not as much as at 1 MSA. However, the application of calcium and boron at the time of the second year of application showed no significant difference to the decrease in contamination of yellow sap on aryl and mangosteen rind.

The time difference between 1 WAA and 4 WAA is still not able to show the effect of calcium application in reducing the yellow sap contamination clearly. The time interval between 1WAA and 4 WAA is thought to be too close to determine the effect of calcium application time on the removal of yellow sap contamination on mangosteen fruit. 1st Class, 1-4 1-4, is the most important time for calcium and boron for plants. The application of calcium and boron at the time of anthesis plays an important role in fulfilling calcium and boron needs to reduce yellow sap contamination.

In addition, the number and level of different fruit growth and development stages in one plant is thought to affect the absorption and translocation rates of calcium and boron to the mangosteen fruit.

Ca^{2+} absorbed in root especially in root elongation zone, located between the root meristem and root differentiation zone (Kurniadinata *et al*., 2017). At anthesis and Stage I, plants also initiate new roots (Marschner, 1995; Matthew *et al*., 2011). Hidayat (2002) and Lijuam Yang *et al*., (2012) explained that the mangosteen rapid root growth occurred before bud break due to an increased need of assimilates to perform high rate of cell division. Development of new roots is an important factor in the mechanism of Ca uptake and translocation from the root to the xylem since Ca^{2+} is mainly absorbed by young root tissue (Himelrick and McDuffie 1983; Marschner 1995; Giuseppe *et al*., 2010). Ca^{2+} can easily pass through endodermis of young roots. Ca translocation is more limited in older roots with well-formed casparian strips; casparian strip will shield the cells and block Ca^{2+} translocation into the xylem.

The experiments showed that anthesis is the best time for Ca application. At this stage, fruits become the strong sink, xylem tissues function optimally, and young roots are already formed and functioning. Application of Ca can be repeated at 4 WAA. Twice application of Ca at anthesis and 4 WAA will increase availability and uptake of Ca into fruit. Ca^{2+} that has been absorbed by young roots since anthesis and 4 WAA will be translocated into the fruit tissues throughout the growth and fruit development stage (Figure 1). The results of this study have provided important information to increase the quality of mangosteen by reducing yellow sap incidence.

Conclusion

From this experiment it can be concluded that:

1. Application of calcium was able to reduce the contamination of yellow sap on the mangosteen aryl fruit reached 53% and, on the rind, reached 46% and improved the quality of mangosteen fruit, without calcium produced plants while yellow sap contamination of 91.66% on aryl and 86% on the rind;
2. Calcium is absorbed and distributed by the mangosteen plant through several mechanisms, namely: through xylem in root and continue to the stem and then to pedicel before absorbed into the fruits. The higher Calcium translocation to fruits is at the rapid fruit growth.

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Status of Growth Hormone and Its Relationship with Vegetative Growth of Mandarin cv. Batu 55 Resulted from Top Working with Several Interstocks

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Abstract

The research was aimed to obtain information on the status of growth hormone and its relationship to vegetative growth of mandarin cv. Batu 55 resulted from top working technique.

The research was done in Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI), East Java, from April to October 2013. A randomized Complete Block Design was used with 10 treatments which were combined between interstocks and top working technique. The result showed that the highest mandarin cv. Batu 55 leaf auxin concentration was found on treatment of KB/S/JC-SK (mandarin cv. Batu 55/interstock tangerine/JC-bark grafting) about 217,17µg/g, while the lowest one was on treatment KB/JC-Ok (mandarin cv. Batu 55/JC-bud grafting) about 9,99µg/g. The highest cytokinin concentration was found on treatment of KB/M/JC-SK (mandarin cv. Batu 55/interstock sweet orange/JC-bark grafting) about 13,38µg/g, while the lowest one was on treatment of KB/Pr/JC-Ok (mandarin cv. Batu 55/interstock lime/JC-bud grafting) about 2,52µg/g. The highest giberellin concentration on treatment of KB/S/JC-Ok (mandarin cv. Batu 55/interstock tangerine/JC-bud grafting) was about 3.122,232µg/g, while the lowest on treatment of KB/P/JC-Ok (mandarin cv. Batu 55/interstock pummelo/JC-bud grafting) was about 277,54µg/g. Furthermore, the highest abscisic acid was found on treatment of KB/S/JC-SK (mandarin cv. Batu 55/interstock tangerine/JC-bark grafting) which was about 6,88µg/g, while it was not detected on KB/P/JC-Ok (mandarin cv. Batu 55/interstock pummelo/JC-bud grafting).

The result of correlation analysis showed that the growth of plant height and number of leave is proportional to the increase of cytokinin and gibberellin concentration but negatively influenced by the concentration of auxin and abscisic acid.

Keywords: citrus, hormone, top working

Introduction

The mechanism of relationship between rootstock and scion is reciprocal which means that the growth of the rootstock will affect the growth of scion and vice versa. The use of dwarf rootstock stimulates apple plants to bear fruit faster due to high amounts of carbohydrates accumulated in the scion. Sometimes the reciprocal relationship does not occur, causing incompatibility that is characterized by death in young plants, inhibition of plant growth, yellowing and falling leaves and asymmetrical growth in the rootstock and scion. To overcome this, a third plant material called an interstocks or an intermediate stem could be used [1].

The interstocks function is expected to be a bridge connecting the rootstock and scion, so that the plant will grow normally as preferred. [2] Interstocks will affect vegetative and generative growth of scion. In citrus, the use of Citrumello interstocks stimulate potentially on vegetative plants growth; interstock Rangpur Lime, Flying Dragon and Troyer have the potential to control plant growth, while the Flying Dragon interstock has the potential to promote flowering and fruiting Nambangan pummelo. The use of different interstocks is able to stimulate the plant growth of Mandarin cv. Batu 55, Tangerine cv Pontianak, and Lime cv. Nimas [3]. Insertion of interstock between rootstock and scion could improve growth and also increase its tolerance to salinity [4].

There are 3 resistance groups to B theobromine in regards of interstock combination [5].

Vegetative growth and fruit production were influenced by the type of rootstock when Flying

Dragon is used as an interstock. On Catania 2 Volkamer Lemon, the Flying Dragon interstock decreased the size of the plant, while on Davis A. Trifoliate rootstock, the Flying Dragon interstock increased the size of the plant [6]. The use of the Flying Dragon interstock also causes differences in vegetative growth in different rootstocks on the scions of grapefruit Star Ruby, Mikhal and Nova tangerine [6]. Lemon which grafted to the interstock caused long plant life, increase fruit production, increase fruit quality and reduce bark thickness in the joint area [7].

Top working is a method of changing plants varieties that already exist in the field with new varieties according to market tastes quickly, without having to turn off the plants [8]. Top working methods can be done by bark grafting, cleft grafting, and bud grafting. The results of top working research have been carried out on grape, avocado, mango, apple, and durian plants [9], [10], [11], [12], [13]. In top working technique, if the plant is pruned too high, the stem left can be used as an interstock. There is an exception for Lemon which is only compatible as an interstock for citrus other than Lemon. Conversely, when the cut is too low, the stem left will be used as the rootstock so that the plant grows as a combination of the scion and rootstock as in grafting in general [14].

Plant growth regulators or phytohormones include auxins, gibberellins, cytokinin's, ethylene, growth retardants and growth inhibitors are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts [15]. Gibberellins control plant growth and fruit development in various ways and at different developmental stages [16], [15]. Gibberellic acid (GA3) and potassium nitrate have a wide range of uses in citriculture [17]. The application of growth regulators has been found to be beneficial in increasing the fruit set in many crops like apple, citrus mango, grapes etc. [18].

The research aimed to obtain information on the status of growth hormone and its relationship to vegetative growth of mandarin cv. Batu 55 resulted from top working technique on several interstocks.

Methodology

The research was done in Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI), East Java, from April to October 2013. A randomized Complete Block Design was used with 10 treatments which have combinations between interstocks and top working technique. Mandarin cv. Batu 55 (KB) as the scion on different interstocks: Pummelo (P), Sweet orange (M), Tangerine (S) and Purut (Pr) with rootstock Japansche citroen (JC), and two types of grafting: bark grafting (SK) and bud grafting (OK). Ten treatments are: (1) KB/P/JC – SK, (2) KB/P/JC – OK, (3) KB/M/JC-SK, (4) KB/M/JC – OK (5) KB/S/JC – SK (6) KB/S/JC – OK (7) KB/Pr/JC – SK (8) KB/Pr/JC – OK and as controls (9) KB/JC – SK and (10) KB/JC – OK, each replicated 9 times.

Parameter observed were:

- Hormones content (auxin, cytokinin, gibberillin, abscisic acid);
- Shoot height;
- Number of leaves.

Analyses

HPLC (*High Performance Liquid Chromatography*) was used to know the leaf hormonal content while relationship between hormonal content and vegetative growth was analyzed using correlation analysis.

Result

The analysis result of leaf hormones of mandarin cv Batu 55 is presented in Table 1.

Table 1. Content of leaf relative hormone auxin, cytokinin, gibberellin and abscisic acid of mandarin cv Batu 55

Treatments	Relative Hormone Content (µg/g fresh weight)			
	Auxin	Cytokinin	Gibberellin	Abscisic acid
KB/P/JC-SK	79,14	nd	1,074,88	4,05
KB/P/JC-Ok	59,93	4,97	277,54	nd
KB/M/JC-SK	142,68	13,38	639,79	3,36
KB/M/JC-Ok	62,85	11,40	417,63	1,77
KB/S/JC-SK	217,17	nd	422,60	6,88
KB/S/JC-Ok	86,21	12,62	2,352,93	2,13
KB/Pr/JC-SK	88,90	nd	514,54	5,67
KB/Pr/JC-Ok	45,31	2,52	2,353,15	1,98
KB/JC-SK	105,87	nd	3,122,23	2,64
KB/JC-Ok	9,99	16,25	3,036,52	3,42

remarks: nd (not detected)

The treatment of KB/S/JC-SK (mandarin cv. Batu 55/tangerine/rootstock JC – bark grafting) had an auxin content of 217.17µg/g, cytokinin (undetectable), gibberellin 422.60µg/g and abscisic acid content up to 6.88µg/g (Table 1). From these data, the content of auxin and abscisic acid is high, while cytokinin and gibberellin are low, compared to other treatments. In plants, auxin plays a role in regulating the development of flower buds and ethylene production [19], [20] while the application of gibberellin acid (GA3) increases fruit set [17]. The higher auxin the faster it's appearance of flower buds and the more ethylene production which will support flowering.

Cytokinin content in grafted plants is related to the rootstock performance and auxin content.

If the rootstock is not vigor, it will stimulate the growth of the scion by increasing the production of cytokinin and decreasing the production of auxin [21].

In KB/S/JC-SK treatment (mandarin cv. Batu 55 /tangerine/rootstock JC – bark grafting), the cytokinin content is low and the auxin is high meaning that the tangerine interstock grows vigorously and disrupts the growth of the scion of Batu 55. Rootstock and interstock can affect the ratio of endogenous hormones on the scion, influence the movement of assimilate and the amount of water circulating from the rootstock or interstock to the scion. Gibberellin content in the treatment of KB/S/JC-SK (mandarin cv. Batu 55 /tangerine interstock/JC-bark grafting) is low. [22]

Higher gibberellin content in plants would induce vegetative bud formation, whereas low content would induce flower buds. The abscisic acid content in this treatment is high, which can be associated with a low cytokinin content, indicating the plants are in the generative phase. [23]

The flowering plant of F1 resulted from the crossing of mandarin cv SoE with tangerine cv Mamuju and Pontianak, higher abscisic acid content and lower cytokinin content were found compared to non-flowering plants.[24] ABA hormone is likely to be one of the key factors in the development of flower buds. From the hormone content of auxin, cytokinin, gibberellin and abscisic acid in this treatment, it shows that the plant leads to the generative phase. This is also, which considers high for citrus and for a generative phase [8].

Shoot height and leaf number observation result is presented in Table 2 [8], while their correlations with hormones are depicted in Figure 1 and 2.

Table 2. Shoot height on mandarin cv Batu 55, 18 weeks after top working

Treatments	**Parameter**	
	Shoot height average	Leaf number average
KB/P/JC-SK	25.14	21.29
KB/P/JC-Ok	55.6	43
KB/M/JC-SK	24.72	20.67
KB/M/JC-Ok	46.33	31
KB/S/JC-SK	17.9	17
KB/S/JC-Ok	58.94	43
KB/Pr/JC-SK	15.79	15.25
KB/Pr/JC-Ok	32.83	22.14
KB/JC-SK	32.25	23.5
KB/JC-Ok	45.13	30.13

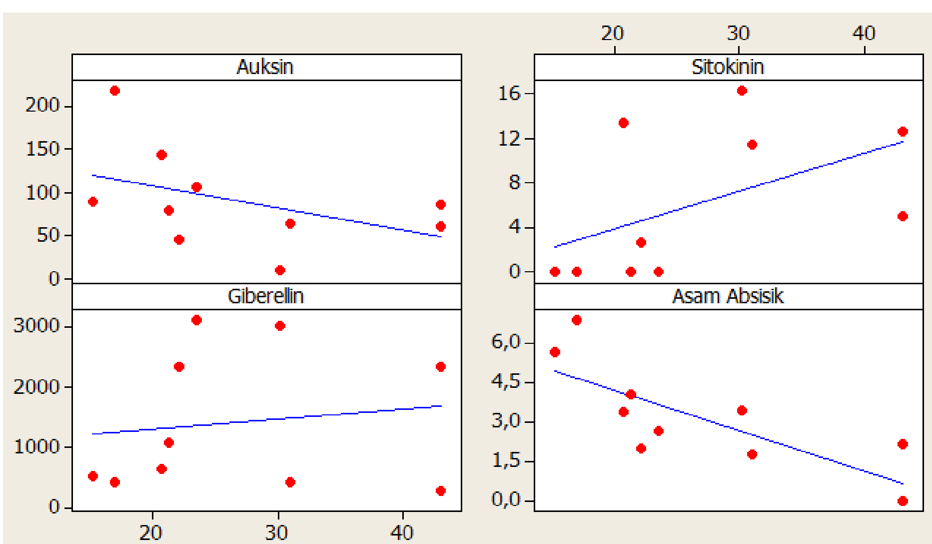

Fig. 1. Relationship between auxin, cytokinin, gibberellin and abscisic acid with shoot height

Based on the correlation analysis above, it can be mentioned that plant height and number of leaves affect the hormone of auxin and abscisic acid negatively and significantly, meaning that the hormone of auxin and abscisic acid in citrus leaves will decrease with increasing plant height and leaf number. The role of auxin hormone in vegetative growth of plants is for cell elongation, phototropism, geotropism and apical dominance, while the role of abscisic acid is for opening and closing stomata, growth and geotropism [20] so that when the plant begins to increase its growth, the requirement for hormone auxin and abscisic acid are also increasing. This results in decreasing content of the two hormones in plants because they are needed to boost growth.

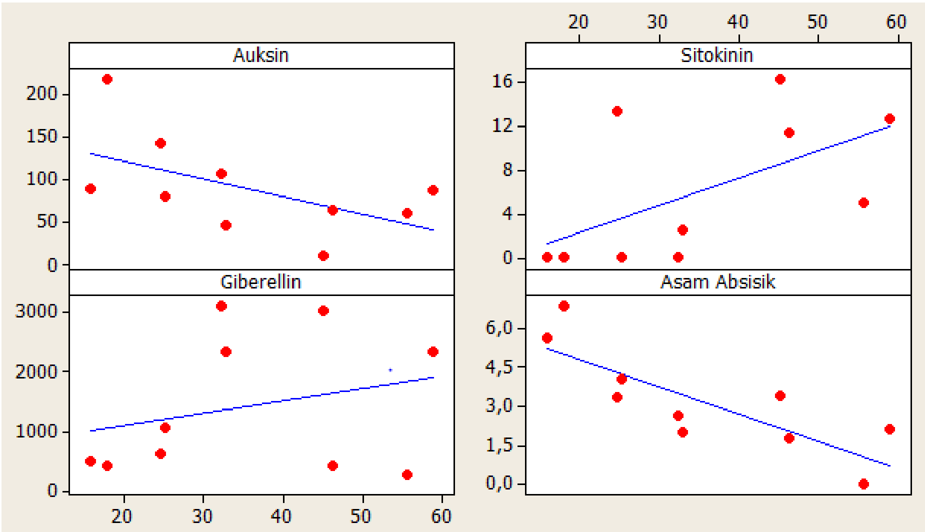

Fig. 2. Relationship between auxin, cytokinin, gibberellin and abscisic acid with leaf number

Contrary to the hormone auxin and abscisic acid, the growth of plant height and the number of leaves is in line with the increase of the content of cytokinin and gibberellin, meaning that if the growth of plant height and number of leaves increases, the content of cytokinin and giberillin hormones will increase as well and vice versa. The real role of cytokinin hormones is in shoot growth [1] and cell division [25], whereas the role of the giberillin hormone is to encourage stem extension, inhibit root formation [1], encourage growth of lateral meristems in leaves and between node [25]. In general, plant hormone is an endogenous factor that is important to regulate all aspects of vegetative and reproductive development of plants and is therefore considered to play an important role in the relationship between roots and shoots. In addition, plant hormones also play an important role in regulating the development of processes and tissues involved in plant responses to various biotic and abiotic stresses [26].

Hormones are produced by one part of the plant and affect other parts. Hormones move in plants from production sites to activity sites. Based on this concept, auxin produced at the end of the bud is transferred to the root which will affect the development, morphology and function of the root [27]. The presence of hormones in the leaves can be used as a basis for knowing the development of plants. The results of leaf analysis of unflowering mandarin plants showed cytokinin content of 38.96µg/g, abscisic acid 0.26µg/g, auxin 4.104µg/g and gibberellin 11.1µg/g while those with flowering had cytokinin content of 19.78µg/g, abscisic acid 23.57µg/g, and the rest hormones were not detected. Hence, plant hormone that plays an important role in the flowering process is abscisic acid.

Conclusion

- Treatment of KB/S/JC-SK (mandarin cv. Batu 55 /tangerine interstock/rootatock JC- bark grafting) showed auxin content of 217.17µg/g, cytokinin undetected, gibberellin 422.60µg/g and abscisic content acid is 6.88µg/g. The auxin and abscisic acid content were high, while cytokinin and gibberellin content was low, compared to other treatments.
- Auxin and abscisic acid hormone contents in citrus leaves will decrease in line with the increase of plant growth and leaf number.
- The content of cytokinin and giberillin hormones will increase in line with the increase of plant height and number of leaf and vice versa.

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Strategies to Increase Tropical Fruit Production and Productivity in Indonesia

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Abstract

There is a high demand of tropical fruits in both domestic and export markets. Indonesia has potential tropical fruit producer in the world. A large variety of tropical fruits are grown in Indonesia like mango, mangosteen, durian, rambutan, banana, papaya, pineapple, and salacca.

Some of them are native to Indonesia such as salacca and mangosteen. In addition, Indonesia leads the world in production of pineapple, salacca, and banana. About 90% of Indonesian fruits production is consumed domestically and 10% is exported to other countries. The objectives of development program of Indonesian tropical fruits were to achieve self-sufficiency and export.

The strategy depend on the existing condition of each commodityy. In general, the strategies could be done by increasing production and productivity through seed production and distribution throughout Indonesia. Indonesian government has declared 2018 as the year of horticulture seedling. Various fruits and vegetables seeds and seedlings are produced in large quantities for distribution to farmers and community. Rapid seed propagation, improving cultivation technology and pests and diseases control are needed to support fruits developing program. The application of Good Agricultural Practices (GAP) is also important in improving fruits quality based on export standards. Increasing collaboration with private sector and planting fruit trees in the border zone are also the focuses of government program in order to make export process to neighboring countries easier.

Keywords: strategy, tropical fruits, production, productivity

Introduction

Indonesia is one of the potential regions of tropical fruits including mango, mangosteen, durian, rambutan, banana, papaya, pineapple, and salacca. High genetic resources, enough labor and land, different harvest season among regions, and wide agroecological zones are the strengths for tropical fruits development. But there are some weaknesses including the problem of quantity, quality and continuity, limited infrastructure and scattered of production areas. Some opportunities of Indonesian tropical fruits are for domestic and export markets, and for bioindustry (food, beverages, cosmetics, others). Competition with imported fruits is a threat that must be overcame.

The largest harvest area of five Indonesian main tropical fruits in 2016 is mango, about 200,000 or 37,6% of the total area. The second rank is banana (90,000 ha/17%), followed by rambutan (81,500 ha/15,3%), durian (80,000 ha/15,1%), citrus (60,000 ha/11,3%), and mangosteen (20,000 ha/3,8%). Although mango is the largest harvest area, the consumption value is smallest (0.007 kg/capita/week). This condition is caused by the consumption of mango which is not evenly distributed throughout Indonesia since most of mango production comes from Java. Bananas and rambutan are the most widely consumed by Indonesian people during 2015 and 2016, about 0.113 and 0.084 kg/capita/week respectively [1].

The popularity of Indonesian tropical fruits in foreign countries are increasingly as indicated by the increasing in export volume. Indonesian Ministry of Agriculture noted, in September 2018, Indonesian fruits export was increased by 24 percent compared to the same period in 2017. This condition showed better quality of Indonesian fruits including mangosteen, banana, salacca, citrus, mango, pineapple and rambutan [2]. Pineapple, mango, banana, mangosteen and durian are the main fruit export commodities (Figure 1). [3]

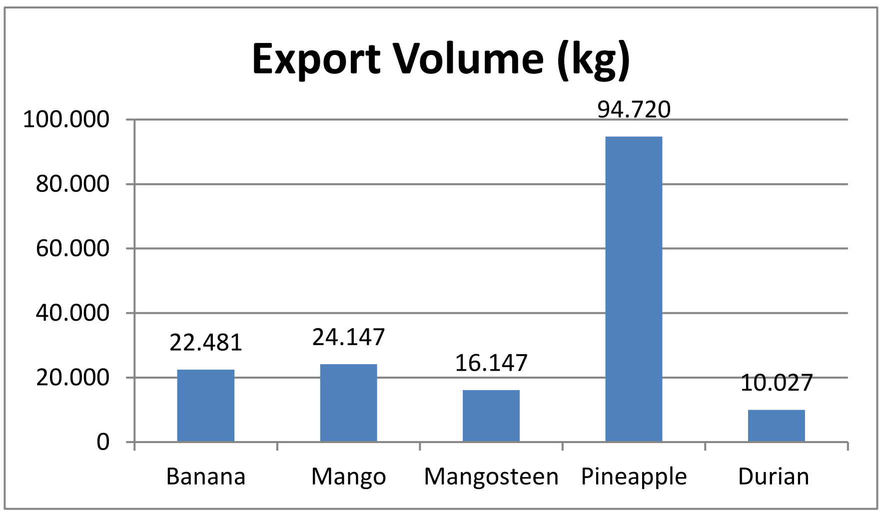

Fig. 1. The top five Indonesian fruit export commodities [3] (Source: Statistik Pertanian, 2017).

Potential of Indonesian Fruit Production

The demand of tropical fruits both for domestic and export market is increase time to time.

Indonesia is great potential producer of tropical fruits in the world [4]. Various types of tropical fruits grow and develop in Indonesia such as mangoes, mangosteen, durian, rambutan, banana, papaya, pineapple, and salacca. Some of them are native to Indonesia such as salacca and mangosteen. Indonesia is the world’s largest country in the production of pineapple and salacca.

Indonesian banana production is the sixth rank after Brazil, mango and mangosteen are the fifth rank after Mexico and Thailand. Compilation data from various sources showed that Indonesia is the largest durian producer in the world, followed by Thailand, Malaysia, Vietnam, Philippines, Brunei and Australia (Table 1).

Table 1. Potential of Indonesian fruits production

Rank	Mango		Banana		Mangosteen		Durian	
	Country	Volume (tonnes)	Country	Volume (tonnes)	Country	Volume (tonnes)	Country	Volume (tonnes)
1	India	18.779.000	India	7.004.019	India	15.250.000	Indonesia	735.000
2	China	4.771.038	China,	2.971.207	China, mainland	4.400.000	Thailand	569.000
3	Thailand	3.432.129	Philippines	23.38.494	Kenya	2.781.,706	Malaysia	302.000
4	Mexico	2.197.313	Ecuador	1.974.865	Thailand	2.650.000	Vietnam	217.000
5	Indonesia	2.184.399	Brazil	1.943.868	Indonesia	2.376.339	Philipine	71.400
6	Pakistan	1.606.091	Indonesia	1.743.028	Pakistan	1.950.000	Brunei	652
7	Brazil	1.417.149	Angola	842.486	Mexico	1.760.588	Australia	60
8	Egypt	1.277.008	Guatemala	732.240	Brazil	1.175.735		
9	Bangladesh	1.161.685	Tanzania	711.045	Bangladesh	945.059		
10	Nigeria	917.617	Mexico	620.675	Nigeria	860,000		

Sources: [5], [6], [7]

Strategies in Increasing Tropical Fruit Production and Productivity

About 90% of Indonesian fruit production is consumed domestically and 10% exported to other countries. The objectives of Indonesian fruit development is to meet domestic demand and to increase export volume. The targets are improving quality, quantity and continuity of tropical fruits to be the largest exporter in Southeast Asia and in the world in 2045. This program is part of the agenda of the 'Orange Revolution', an Indonesian program of tropical fruits production.

The strategy in developing tropical fruits production could be done through the development of varieties suitable to market preferences based on comercial scale. Rapid seedlings propagation, improved cultivation technology and pest and disease control are needed to support the program.

The application of Good Agricultural Practices (GAP) is also important in improving productivity and quality of fruit based on export standards. Increasing collaboration with the pivate sector and developing the fruit crops in the border area is also focus of the government to make export process to neighboring countries more easier. The objectives of Indonesian tropical fruit development program is to achieve self-sufficiency and increasing export volume.

Strategies in Increasing Mango Production

Indonesia is one of the top ten mango producing country, the fifth rank after Mexico [5]. But Indonesian contribution to the world market is very small. The main problem in mango development including: 1. The existing varieties are still diverse and not in accordance with consumer preferences, 2. Inadequate postharvest handling, 3. Continuity of fruit production is not guaranteed, 4. Limited of commercial scale orchards. In addition, the low consumption of mango is caused by not evenly mango distribution throughout Indonesia since most of mango production are came from Java. The level of damage of the fruits is high and the price becomes expensive.

Therefore the development of the area outside of Java is absolutely necessary, especially in areas that have suitable agroclimate conditions. It is necessary to develop management models that can solve these problems, including capital, market, technology transfer and others.

The objectives of Indonesian mangoes development is to increase consumption per capita and to increase export volume. The strategies could be done through the supply of plant material in the form of seedlings to support the development of new commercial areas, replacing non commercial varieties with commercial varieties through top working, and applying cultivation technology according to SOP and GAP. Two superior varieties, Agrigardina 45 and Garifta have potential to be developed [8].

To accelerate mango development, it is necessary to collaborate with private sectors by establishing satellite orchard, and developing water storage for irrigation facility. Top working technology is suitable to replace the old varieties with commercial varieties. Quality improvement, technology off-season, and postharvest technology are also needed. For increasing mango production about 330,000 tons or equivalent to Mexico, it is necessary to plant new area for mango crop about 30,000 hectares. If the development program directs to increase consumption/capita from 0,34 to 3.25 kg/capita/year, it is necessary to produce 873,679 tons or need to develop new area of about 79,425 hectares.

Strategies in Increasing Durian Production

Indonesia is the largest durian producer in the world (Table 1). The problem is the diversity of Indonesian durian is very large and comercially planting area is still limited. Developing durian orchards in concentrated areas is needed and require large quantities seedlings or planting materials. Development area of durian throughout Indonesia to achieve the ideal production is about 57.500 hectares.

To fulfill the needs of durian seedlings and to facilitate seedlings transportation easier, it is important to decentralize seedling production in development area. At each location, the number of seedlings produced based on the number of mother plants or scions they have. Collaboration with private sectors and seed producers could accelerate the process of seedlings supply. Seed certification institution plays a role in guarding the quality of seedlings during production process until distribution. The selected superior varieties recommended are Pelangi Atururi, Salisun, Matahari. Sitokong, and Lai Mahakam.

Strategies in Increasing Banana Production

The success of banana development in Indonesia requires an active roles of all parties, including governments, seed producers, or seed companies, farmers as main actors; and producers of banana producers such as farmer groups or a combination of capable farmer groups that providing large-scale business land and merchants who market products.

Several strategies can be implemented in bananas development such as: 1. Fostering and establishing seed institutions, production facilities, finance, product handling and marketing, 2. Development of seed networks by establishing the seed production centers in several areas representing the surrounding provinces, 3. The application of banana cultivation technologies starting from the selection of superior and commercial cultivars, preparation of planting materials, fields, planting, maintenance, harvesting, until fresh handling and processing of products.

Currently cooking commercial banana cultivar being developed is Kepok Tanjung which is used to control bacterial wilt that attacks Kepok in almost all of part of Indonesia. Whereas for dessert banana developed is Barangan.

Strategies in Increasing Mangosteen Production

Mangosteen is one of the commodities that will be developed on a commercial scale. In 2016 Indonesian mangosteen production is about 176.000 tons with an area of 22.377 ha, and exports about 138.000 tons. This production was lower than Thailand with production of 2,650,000 tons, and export volume of 195.638.000 tons [9]. Increasing the production and export of Indonesian mangosteen, can be done through expanding the area and improving cultivation technology. To increase the production of Indonesian mangosteen equivalent to Thailand, the increase of planting area is needed from 22,377 ha to 31.723 ha or an additional area of about 9,346 ha. For this purpose about 934.600 seedlings are needed.

Seed Production and Distribution

One of fruit development problems in Indonesia is limited of plant materials (seedlings) to be planted. This condition are caused by several factors: 1). Limited number of parent trees as a source of seeds or scion, 2. Limited of propagation techniques, 3). Plant tissue culture technology is still limited, and 4). The seed availlability depend on fruit season. The availability of good quality seedlings is required to increase fruit production. Most of the new variety released as single trees.

Indonesian Ministry of Agriculture has declared 2018 as a year of horticulture. Various seedlings of fruit commodities are produced in large quantities to be distributed to farmers and the communities. Indonesian Tropical Fruits Research Institute (ITFRI) as executing agency has produced tropical fruit seedlings and distribute to stakeholders. The tropical fruits seed production program consists of: 1). Increase the number of parent trees through determination of duplicate trees, DNA analysis, registration of parent trees and developing Block Fondation of Scion. 2).

Accelerate the growth of seedlings and 3). Provision of seed production facilities and infrastructure. In 2017 ITFRI will produce about 971.000 tropical fruit seedlings (Table 2.) consist of mango, mangosteen, durian, banana, salacca, papaya, and breadfruit to distribute for farmers and growers.

Table 2. Tropical Fruit seed Production by IAARD 2017-2018

N0	Commodity	2017	2018	TOTAL	Estimation new planting area (ha)
1	Mango	370.000	-	370.000	3700
2	Mangosteen	8.500	35.000	43.500	435
3	Durian	105.000	47.000	152.000	2530
4	Banana	12.000	10.000	22.000	220
5	Papaya	215.000	30.000	245.000	2450
6	Salacca	30.000	-	30.000	300
9	Sukun	20.000	25.000	45.000	450
10	Avocado	-	3500	3.500	350
	Total			911.000	910

The mechanism of seed production conducted by ITFRI is carried out through the development of seed industry in collaboration with the Directorate General of Horticulture, seed breeders, farmer groups, seed growers and seed certification institution. Provision of seedlings in large quantities and short periods is carried out through tissue culture technology. Currently there are many commercial tissue culture seed companies, especially for bananas. The truth and purity of cultivars produced is a necessity through the establishment of a registered parent trees.

To accelerate supply of mango seedlings for development program, Indonesia Ministry of Agriculture provides the extension seedlings of superior varieties and distributed to farmers and communities. The seedlings production is carried out by IAARD at the Experimental Farm and in collaboration with mango seed growers in Pasuruan Regency, East Java (Figure 2). The Seedlings are produced through strict supervision in accordance with the Standard Operating Procedure (SOP) by Seed Certification Institution. This collaboration provides benefits to both IAARD and seed growers. IAARD has benefit in terms of: 1. Acceleration of the seed production process, 2. Acceleration of development and dissemination of the new varieties, 3. Overcoming the limitations of land and labor for large-scale seed production, 4. Developing good synergy between government institutions and communities in implementing development of fruit commodity. The benefits received by seed growers are: 1. Availability of new employment opportunities, 2. Increasing knowledge in mango seed production through technology transfer, 3. Increasing seed growers income.

Fig. 2. Collaboration between IAARD and seed growers in production of mango seedlings

Application of *Good Agricultural Practices* (GAP)

Application of GAP system will give benefit both to humans and environment. GAP certified products certainly have their own guarantees for consumers. Through GAP, everything is recorded since the selection of the orchard until harvesting. The application of GAP for fruit commodity in Indonesia is based on the Regulation of the Ministry of Agriculture No. 61 /Permentan/OT.160/ 11/2006, November 28, 2006. GAP mentoring is needed to improve knowledge, attitudes and skills of farmers in fruit cultivation to produce quality and safe consumption products. After implementing GAP, producers should also implement Good Handling Practices and Good Manufacturing Practices. [10]

Fruit Development at the Border Area

One of the other focuses of the Indonesian Ministry of Agriculture in 2018 is to support the development of agricultural commodities in the border area. The aims are to realize food sovereignty and become a world food storage country by 2045. Kepulauan Riau Province is one of the target of border areas in developing fruits commodities. This area is directly contact with neighboring country of Malaysia, Singapore, Vietnam and Cambodia. This area is very potential because it includes in international free trade zone.

To increase productivity of tropical fruits in the border area, IAARD has designed integrated activities between the IAARD institutions and the local government, the private sector. This program becomes more efective by collecting financial, facilities and human resources. Fruit Development program in the border zone of Kepulauan Riau Province is very prospective to fulfill the fruits needs of local community and for export to Malaysia and Singapore. The activities that have been carried out are established mango and dragon fruit orchards, developing parent trees orchard of superior fruit varieties, and assisting in propagation and cultivation of fruit trees.

Collaboration with Private Sector

Indonesian population growth is still high, about 1.4% per year. In addition the increasing of the new middle class has a significant effect on increasing the food needs both in quality and quantity. The development of national food security in the future must be able to support food availability for the community, based on local genetic resources. The fruit development model will be carried out by the central and regional governments and universities through the collaboration of 4 pillars (Academics, Private Sector, Government and Community).

The key resources are IAARD as the technology owner, private sector and farmers or the community as producers. The private sector act as a consumer as well as producer of fruits products. The government acts as a regulator. Every partner must accelerate the business to be profitable. Key activities consist of seed production, in collaboration with breeders, fruit production and marketing by private and plasma farmers. Value preposition is forming a fruits orchards garden with good productivity, quality, and continuity. In addition, free of pests and diseases and safe for consumption. Main customer segments are export markets (United Arab Emirates, China, Europe) and modern local markets. One example of collaboration with the private sector is the collaboration between ITFRI and PT. New View Glen Falloch, Banyuwangi East Java, in developing papaya. The papaya orhard product has reached the stage of commercialization.

Fig. 2. Collaboration between IAARD and private sevtors PT. New View Glen Falloch in papaya production

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Citrus in Indonesia: Production Perspective for Market Development

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Abstracts

Citrus is one popular ingredient in the dishes and drinks of many countries including Indonesia. The production of domestic citrus in the country has fluctuated due to the attacks of various pests and diseases, especially Hunglongbing (HLB). The citrus rehabilitation program has been carried out to alleviate the problems in more than 50 citrus production centers. Implementation of the improved technologies accompanied by standard food safety regulation based on GAP and SOP were dedicated to increase higher quality and quantities of domestic citrus in many market levels. The implementation of the program was expected increase consumer preferences to domestic citrus and penetration of domestic citrus to wider international market segments.

Keywords: Citrus, production, rehabilitation program, productivity, market segment

Introduction

Citrus is one of economically important fruit commodities in Indonesia. Citrus positioned at the third ranks after rambutan and banana as the most domestically consumed fruits in 2015 and 2016. People consumption reached 0.06 kg per week in 2015 and increased up to 0.09 kg per week at 2016. Citrus also ranked at the first for the average fruit expenditure of the Indonesian (in rupiah), both in the village and in the city based on the National Social and Economic Survey household Consumption and Expenditure Supplement [1].

In terms of international trade, Indonesia has higher import than export values. Among South East Asian countries, Indonesia was considered the second largest citrus importer after Malaysia with the increasing trend up to 11% per year [2]. These conditions were triggered by several factors, like increase of consumer demand, insufficient supply by domestic production and reduction of citrus tariff barriers [3].

Citrus is usually grown on high land and lowland area including swampy area under dry and wet climates. Citrus plantation in Indonesia was characterized by small-area holding farmers, scattered and forming production centers. Recently several bigger growers have been established with the coverage of more than 100 ha. The total area of citrus plantations in 2017 were recorded 56,757 ha with total production of 2,295,310 tons and productivity of 40.4 ton/ha [4. In general, the proportion of citrus varieties grown by citrus grower are 80% of tangerine, 10% of mandarin, 6% of pumeloes and 4% other, like lemon, lime and *citrus hystrix*.

Situation of Citrus Production in Indonesia: An Overview

Citrus production in Indonesia has been fluctuated since the last decade. Recorded almost 2.4 million tons at 2008, the production diminished until the least volume of 1.5 million tons in 2015.

The positive production trends then recovered up to present at 2.2 million tons at 2017 (Indonesian Statistic Bureau 2017b). The fluctuated citrus production was reported due to various problems especially pest and disease attacks especially Huanglongbing (HLB) in several main production centers [5].

HLB is caused by negative gram phloem limited fastidious prokaryotic bacteria, *Candidatus Liberibacter asiaticus.* The disease was the most devastated citrus disease in the world. The disease may spread through tree canopy, causing decline and death of the plant [6]; [7]. In Indonesia, the disease was also known as citrus vein phloem degeneration (CVPD) which had destroyed most citrus plantation in during 1960s to 1970s. Misidentification on the cause of the disease at that time had made control efforts fail in decreasing the disease spready and economic losses. In the middle of 1980s, the government launched the program of 'Citrus Rehabilitation Program' that focused on the revitalization of the production centers through massive production and distribution of disease-free (healthy) planting materials. During 1990s to middle of 2010s, HLB control program had been designed more comprehensive after the successful identification of bacterial pathogen and molecular detection of HLB. The program was implemented based on (1) the large-scale production of disease-free planting material, (2) introduction of improved orchard management procedures, and (3) development of efficient pest and disease control measures to protect new plantings from reinfection [8].

The revitalization program on citrus production centers increased confident among the growers to reestablish citrus plantation through healthy planting material and technology guidance by government. The first released rehabilitation program was carried out at East Java and Bali during 1991-1992 with initial releases of around 1 and 0.5 million planting materials in East Java and Bali, respectively. As the results, former production centers in Bali and East Java that previously more than 4 million trees had been cut down due to the disease [9], had positioned these areas at the first and third ranks of the citrus production centers today (Table 1).

Table 1. Citrus production by province in Indonesia during 2013-2017

Province	2013	2014	2015	2016	2017	2017 Rank
East Java	514855	568775	480396	837370	898279	1
North Sumatra	326322	500244	483006	459149	435454	2
Bali	140581	98524	129136	83739	168383	3
West Borneo	154305	187016	147371	124202	126502	4
South Borneo	109100	129526	112301	108210	123874	5
West Sumatra	40522	55180	64548	86786	102733	6
South Sumatra	14880	10171	16141	15231	39756	7
West Sulawesi	25209	25506	58289	42539	34930	8
Southeast Sulawesi	53421	37034	51231	58216	32704	9
West Java	29487	29539	54127	27160	24594	10

Source: [10]

The national implementation of citrus revitalization program has increased the harvesting area and national production that reached the peak at until 2007-2008. Different situation was detected on consumer demand during that time. The increase people income affected fruit consumption pattern. The consumer demands have increased significantly since then up to this moment [11].

The positive indication of consumer demand, on the other hands, was not accordingly followed with the domestic citrus production. The increase of the production was then considered stagnant due to the diminishing harvesting area (conversion of agricultural land). The domestic citrus supplies were not able to coupe up with the increase of market demand, especially those from middle class consumers. Imported citrus from China, Australia, Pakistan, and USA then have become the way out to fulfill the lack of products with an increase of 11% per year. While export volumes were still less than 450 tons with the targeted countries of Malaysia, Brunei Darussalam and Middle East.

Recent Issues in Citrus Agribusiness

Huanglongbing (HLB) disease still becomes the main threat in most production centers. Most previous growers were still in doubt to replace the existing crops with citrus as the main business.

The program in HLB control through healthy planting material production and distribution were then also dedicated based on local government requests that are interested to establish a new citrus production center, like in Batang, Central Java.

Through induction program to local governments, the planting area was expected to increase.

Thus, the harvested area of citrus is also projected to increase in the rate of 4.4% per year and by 2020, total harvest area is expected to be 64,592 ha. With the establishment of respected areas, the production would be able to reduce the import and increase export volumes.

Indonesia ranks at the 3rd for mandarin and tangerine types producing countries in the world after China and Spain. The mandarin and tangerine production from China was higher that reached 17.15 million tons, compared to Indonesia that produced only 2.2 million tons per year [12]. For other citrus types, the domestic production was even smaller. Mandarin/tangerine was more preferred and consumed in fresh, while orange was in a processed form by Indonesia consumers [13]. These indicated that the expansion of planting area of both citrus types still had feasible domestic market in Indonesia.

Unlike other plantation crops, citrus orchards in Indonesia are scattered, covering small area owned by individual growers, yet located in the same agroecological zone. Cooperation and coordination among growers in handling the production system were absent. Each grower has different procedure of cultural practices, plant maintenances and harvesting. These conditions bring about the difficulty in the implementation of thorough integrated farming in the production area [14]. The technology transfer was hampered and the improvement of production system in large scale run slowly.

Grower sells the citrus product to the third parties and these usually become the only communal practices for product marketing. Since growers did not have direct access to market, they were less responsive to the price change of product in the market [15]. These conditions also led to the weak bargaining power of citrus growers to get competitive price in harvest season.

Citrus Improvement Program

Citrus improvement program was designed to improve productivity in production area through agroecological and innovative technology approaches. The programs were cooperative activities between local government and Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI). ICSFRI is a technical implementing unit that is mandated and designed for citrus and subtropical fruits research and development in Indonesia. The institution has more than 250 accessions of citrus collection and released more than 50 superior citrus varieties. The varieties have now been disseminated at 50 regencies in 28 provinces in Indonesia for the establishment of citrus agribusiness zone. The program is called Integrated Management of Health Citrus Orchards (IMHCO) that covered certified citrus nursery stocks, Bujangseta (technology to produce citrus fruit year around and harvesting), top working and post-harvest proper handling.

Success of citrus plantation begins from healthy and qualified planting materials. In response to the respected situation, ICSFRI with the certified ISO 17025-2015 testing laboratory and ISO 9001-2018 elite planting material production unit has a standard procedure in indexing seedling health and producing disease-free planting materials. The mother stock plant was indexed for bacterial and systemic disease containment. The disease-contained stock plants were destroyed, while citrus disease-free mother plants were massively propagated using *in vitro* procedures. After propagated, the plants were then kept under controlled environment in the screen house and served as the primary source scions. The mother plants were regularly indexed for systemic and bacterial diseases to ensure the quality of seedlings produced from these stock plants [16]. The derived seedling derived from stock plants were produced through grafting methods of selected scions into rootstocks. The certified planting material were manufactured in polybags with blue label certificate that indicated the true to variety and free from systemic pathogens (CVPD/HLB, CTV, CEV, CPs, CVEC). Qualified planting materials would produce uniform and robust citrus trees.

The maintenance of citrus will also be more efficient. With proper maintenance, the productivity and fruit quality will be expectedly higher and the production period will be longer.

The average of national productivity of citrus plantation was counted only 15-17 tons per hectares at present from the potential of 30-40 tons per hectare. HLB is considered to be one of the main reasons behind these facts. The disease is transmitted by insect *Diaphorina citri* and/or through vegetative and generative propagations [17]. Under IMHCO program, the control of HLB were designed through several technology components, i.e., (1) Planting certified and disease-free planting materials, (2) controlling HLB-transmitting insects using integrated pest management system, (3) Sanitation of plant orchard, (4) Proper plant health maintenances and (5) Consolidation of orchard production system in targeted development area [18]). Implemented in some areas, IMHCO program sounded positive results. IMHCO induced higher orchard productivity in several production areas such as in Banyuwangi, East Java. The growers in these areas enabled to prevent HLB incidences and gained the productivity of 10-12 tons per hectare of Tangerine in extreme drought conditions [19]. IMCHO is more effective when implemented to the new development areas or those that has been rehabilitated and free from of citrus infected plants with the minimum radius of 5 km.

Rehabilitation of citrus orchard were also carried put through top working methods. Adapting the fast changes of consumer preferences, growers enabled to change the old existing variety with modern and more commercial ones. Top working method was also dedicated for rejuvenation of declined-plants with more potent productive plants [20]. The implementation of the respected methods has increased grower income in Dau, Malang East Java. Growers in this area replaced Orange (Baby Java) with more commercial Mandarin variety of Batu 55.

The harvesting season of citrus in Indonesia usually started from February to September with the peak production during May to July. During these periods, the product prices were usually not profitable. On the other hands, citrus fruit were also scarce and expensive when not in the production period. To overcome such situation, ICSFRI introduce ‘Bujangseta’ technology referring to plant maintenance and induction for bearing fruits all year round. The principle of the respected technology is integrated management of canopy, nutrition, and pest. Maintained under Bujangseta system, citrus trees were able to produce fruits consecutively every 2-3 months (5-6 harvesting period per year) without any decrease in plant performance and health. Producing fruits year-round ascertained fruit availability outside the common harvesting period with competitive price and product quality at the market [21].

Implementation of GAP/SOP and Orchard Certification

The concern of consumer safety to consume agricultural products also become important issues in Indonesia. Sufficient food supply, distribution and ease to access are not the only inevitable cases, yet product quality also become consumer awareness and determined product survival in the market. At production site level, the certification 'PRIMA' was dedicated to ensure the production system facilitating all necessary approaches in producing safe, healthy and standardized fruit quality agricultural products [22].

PRIMA certification was divided into three levels, i.e., 1, 2, and 3 respectively. PRIMA 3 considered that the orchard enabled to produce safe agricultural product to be consumed. PRIMA 2 certified not only on product safety, yet on product quality as well. The highest level, PRIMA 1 required the growers to fulfil all the elements in food production requirements, i.e., safe to be consumed, qualified products and the producing processes are environmental friendly. The certificate would be given after consecutive assessment and monitoring on the implementation of Good Agricultural Practices (GAP) principles and Standard Operating Procedure (SOP) by Local Food Security Authority, Agency of National Food Safety. Agricultural products produced by certified orchards have bigger opportunity to sell in any market levels and wider international consumer acceptance.

Table 2. Number of registered fruits orchard in 2015-2018

No	Commodities	Number of orchards
1	Mangosteen	431
2	Salak (snake fruit)	264
3	Citrus	65
4	Dragon fruit	45
5	Banana	27
6	Others	23

Source: Indonesian Ministry of Agriculture (2018)

Marketing

Most of citrus growers still relied on the traditional culture method, producing/harvesting citrus once a year in huge quantities. These practices in almost all case, have made the product price uncompetitive. Growers were always put in difficult and weak bargaining position in achieving higher price regardless the fruit quality produced. On the other hands, consumers also have difficulties in finding the citrus fruits especially when outside of harvesting period. During these periods, imported citrus become the alternative products due to the lack of domestic citrus [23].

The volume and value of imported citrus increased by the time, though it was still considered less than 10% from the total production. The availability of these products in any seasons may shift the consumer preferences and threaten domestic growers.

During the peak harvesting period, growers usually take and sell the products to big cities expecting higher prices. The premium fruits usually were selected for these market levels accompanied by higher price. The premium class citrus fruits were then less available at the surrounding production area. The majority of citrus fruits circulation are in big cities such as Jakarta, Surabaya, Medan, Makassar, Bandung, Semarang, Balikpapan, and other cities.

Conclusion

Citrus production in Indonesia has been fluctuated since the last decade due to some constraints in production level. The harvest area increased yet the productivity still needs technology guidance to boost the national production. Several programs have been introduced i.e., disease-free planting material distributions, HLB control programs, rehabilitation of declined-citrus orchard trough top working methods, and plant maintenance and induction for bearing fruits all year round 'BUJANGSETA'. Implementation of the improved technologies accompanied by standard food safety regulation based on GAP and SOP were expected to increase consumer preferences to domestic citrus products. Domestic fruits enable to fulfill all market levels with sufficient quantities, quality and accessible prices due to year-round availability.

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The Efficacy of Ethanol Vapor in Reducing the Deterioration and Extending the Shelf Life of Strawberry Fruits

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Abstract

Strawberry fruit has high deterioration rate since the fruit is very susceptible to physiological changes, decays, physical damages and water loss. The efficacy of ethanol (EtOH) vapor to reduce the deterioration rate and extend the shelf life of strawberry fruits was investigated by impregnating 0 ml, 3 ml, 6 ml, 9 ml, and 12 ml of EtOH in 5 g of carrageen, and placed in tea sachets. The sachets were then put on the basal of the plastic trays on which 250 g of fruits were placed, and then covered with stretching plastic film LDPE. The fruit packages were stored at temperature of 5±1 °C and 25±1 °C. Control fruits were also provided as comparisons. The results showed that the slow releases of EtOH vapor from the sachets were able to reduce the spoilage intensity and weight loss of the fruits significantly. The vapor also maintained the color and texture during storage of the fruits compared to the control fruits and without treatment (0 mL EtOH).

Storage at 5±1 °C, the higher the volume of EtOH, the higher the hardness and the lower the spoilage of the fruits. Storage at 25±1 °C, however, the control fruits and those treated with 0 ml EtOH were spoilage of about 90% and 85%, respectively at day 3. While at the same time, the spoilage ranged of 10-15% for the fruits treated by EtOH vapor and the panelists still favored the fruit aroma.

Keywords: Strawberry, ethanol vapor, slow release, postharvest shelf life

1. Introduction

Strawberry is a high-value fruit that is become favorite and increasingly consumed by people since its riche's vitamin C, minerals, attracting fruit color and volatile aromatics. The fruit is non-climacteric; therefore, it has to be harvested in the fully ripe stage to get the best eating quality.

Strawberry fruit, however, is among the most perishable produce as it has high respiration rate, thin dermal tissue, soft and watery ground tissue; therefore, very susceptible to water loss, mechanical damage, and fungal decay. Those perishable characteristics lead to short commercial shelf life, just in hour basis if it is stored in the room temperature.

The most common method that commercially used technology to extend the shelf life is by storing the fruits in the low temperature that is close to 0 °C. Since the low temperature in supply chain system is somewhat difficult to maintain or in order to get more extensional shelf life, the additional technologies, which are complementary to this low temperature, have been used to maintain the quality. The effect of atmospheric gases of O_2 and CO_2 in reducing the physiological deterioration has been reported by many researchers [1], [2], [3], [4], [5], [6], [7], [8]. The combination of low oxygen concentration (0.5%, 1.0% and 2%) and elevated CO_2 (10%,15% and 20%) reduced the respiration rate and the production of ethylene of "Selva" strawberry stored at 2 °C [9]. The concentration of 1% O_2+15% CO_2 and 0.5% O_2+20% CO_2 caused the EtOH accumulation which was still detected at the concentration above 100 ppm in the juice of the strawberry after the fruits were transferred in the room temperature. The residual EtOH was expected to maintain the firmness and the color of the fruits and did not significantly affect the color of juice and skin, titratable acidity, total soluble solids, and ascorbic acids. The formation of EtOH may occur due to anaerobic fermentation [10]. EtOH compound has also been reported to retard the ripening of tomato fruit [11], [12].

EtOH is a volatile compound that has biological potencies in slowing the deterioration rate of the fresh horticultural product. Endogenous or exogenous EtOH had been reported to delay the ripening of whole and cut tomato fruits [13], [14], [15], [16]. EtOH concentrations that inhibit the ripening process in tomatoes are generally higher than those naturally present in fruit tissue [15].

Inhibition to ethylene and CO_2 production are thought to be the mode of action of ethanol in retarding the ripening processes [13]. The opposite result is obtained that EtOH vapor stimulates the ripening of kiwifruit [17], and increases the sensory quality of blueberries, tomatoes, and pears, including increasing sugar content, sugar-acid and flavor ratio [18].

Another beneficial effect of EtOH is due to its antimicrobial properties. In vitro experiment indicated that EtOH vapor has germistatic effect on *Rhizopus stolonifer, Penicillium digitatum, Colletotrichum musae, Erwinia carotovora,* and *Pseudomonas aeruginosa* [19]. Other findings indicated that 30% EtOH (v/v) improved the in vivo efficacy of the Benomyl-DCNA in controlling the growth of brown rot (*Molinea fructicola*) in peaches [20]. Lemon orange inoculated with *P. digitatum* spores and dipped in 10% EtOH at 45 °C for 150 seconds significantly reduced decay by microorganisms, and its effectiveness was comparable to immersion into a fungicide 1,000µg/ml imazalil at 25 °C for 60 seconds [21]. It was also reported to delay *P. digitatum and P. italicum* infection on oranges [22].

The recent research reported that exposing ethanol vapor generated from 4 ml/kg strawberry fruits, then cut the fruits to become 4 wedges each and stored at 4 °C for one week, reduced the increasing of weight loss and the declining of firmness, and suppressed the microbial growth [23].

This article, however, reports the efficacy of exogenous ethanol vapor continuously exposed to the strawberry fruits in modified atmosphere packaging during storage at room temperature (25$\pm$1 °C) and low temperature (5$\pm$1 °C) in reducing the deterioration rate and extend the shelf life.

2. Materials and Methods

EtOH (>99.5% purity) used in this research was purchased from Sigma-Aldrich. Different volumes of 0 ml, 3 mL, 6 ml, 9 ml, and 12 ml EtOH were impregnated into matrixes of 5 g carrageen added with 20 ml distilled water and then put in the tea sachets. The impregnated EtOH in the sachets was individually sealed in the plastic pouches and put in the icebox before using for the experiment. The strawberry fruits (Rosalinda cultivar) were directly harvested at the farm located in the Bedugul of Bali. The harvested fruits were brought into the packinghouse near by the farm. The fruits were selected, and the fruits without defects, mechanical damage, about 80-90% red color and fresh-green calyxes were used for the experiment. About 250 g fruits (17-20 fruits) as an experimental unit were put on the plastic trays on which the impregnated ethanol in the sachet was placed (on the basal of the trays). The trays were then sealed using plastic stretching film LDPE. The treated fruits were stored at the room temperature (25$\pm$1 °C) and low temperature

(5±1 ºC). Slow release of EtOH vapor was expected during storage. Control fruits were provided at the two different temperatures as comparisons.

2.1 Spoilage intensity and weight loss measurement

The intensity of spoilage was measured on the treated strawberry fruits that were specially prepared for this measurement at day 3, 6 and 9 of storage. Every time of measurements, the plastic films covering the plastic trays of the unit experiments was opened, and the spoilage of the individual fruits was rated as shown in Table 1. A strawberry fruit, which was spoilage >25%, was assumed unmarketable or inconsumable; therefore, the maximum rating was six. The intensity of spoilage on the experimental unit was then measured using the formula below [24].

Table 1. The individual spoilage of the strawberry fruit and the rating

Individual spoilage (%)	Rating	Individual spoilage (%)	Rating
0	0	16-20	4
1-5	1	21-25	5
6-10	2	>25%	6
11-15	3		

$$IS\ (\%) = \frac{\sum(\boldsymbol{n} \times \boldsymbol{v})}{\boldsymbol{N} \times \boldsymbol{V}} \times 100\% \qquad (1)$$

Where, IS is the intensity of spoilage, N is the number of fruits in the experimental unit, V is the maximum rating, n is the number of fruits in the specific rating, and v is the rating of the spoilage.

The weight loss of the fruit was measured continuously for the similar treated-packaged strawberry fruits at day 3, 6 and 9 days of storage by using the formula below. The fruits which were >25% spoilages were discharged from the package by incising the cover of the plastic film.

After discharging the incised plastic film was covered by a cello tape.

$$WL\ (\%) = \frac{Wo - Wt}{Wo} x100\% \qquad (2)$$

Where WL is the weight loss, Wo is the initial weight, and Wt is the weight of the unit experiment at the time of measurement during storage.

2.2 Color Difference

The color of the skin of strawberry fruits was measured using a colorimeter (Minolta CR-400 Chroma Meter). The L*, a*, and b* color system were involved in this measurement. The color differences between the treated strawberry fruits and the control fruits during the storage were determined using the formula below [25].

$$\Delta E = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*} \qquad (3)$$

Where; ΔE is the total difference of the color, and $\Delta L^* \Delta a^* \Delta b^*$ are the color difference between the L^*, a^* and b^* values of the treated fruits and the control fruits.

2.3 Firmness

The firmness of the strawberry fruits was measured on the whole individual fruit using texture analyzer (TA. XTplusC, USA). The texture of three different fruits from each unit experiments were measured using the 6 mm diameter cylinder probe. The probe was set at 5 sec speed, 10 mm depth, and the firmness was in the unit of Newton (N).

2.4 Vitamin C and Total Soluble Solid

The concentration of vitamin C was measured using iodometric titration [26] involving the formula below.

$$VC = \frac{Vi \times 0.88 \times 100}{W} \qquad (4)$$

Where, *VC* is vitamin C (mg/100g), *Vi* is the volume of Iodine 0.01 N (ml) used for titration, *df* is the dilution factor, and *W* is the weight of the sample (g). The measurement of the total soluble solids of the flesh of the strawberry fruit was conducted using a digital refractometer (Misco V 1.04 Palm Abbe II, USA).

2.5 Preferences of the Aroma, Taste and Color

The organoleptic preferences of aroma, taste of the flesh strawberry fruits was determined by 15 untrained panelists. The fruits were each cut into four longitudinal parts and provided for the panelists. The panelists were asked to test the aroma and the taste of the strawberry with five-point hedonic scales, where 1= strongly dislike, 2 = dislike, 3 = neither like nor dislike, 4 = like, and 5 = extremely like.

2.6 Statistical Analysis

Data gathered from the experiment were analyzed statistically using analysis of variances. If there was a significant difference of the variance due to the treatments or their interactions, the comparison among the means was performed using Duncan's Multiple Range Test (DMRT).

3. Result and Discussion

3.1 Spoilage Intensity and Weight Loss

Analysis of variance of the data indicated that the slow release of EtOH vapor in the modified atmosphere packaging of strawberry fruits interact significantly ($P \geq 0.05$) with the storage temperatures in their effect on the spoilage intensity of the fruits during storage. On the Figure 1 clearly shown that the EtOH vapor of E3-E12 significantly reduced the intensity of spoilage of the strawberry fruits measured at day-3 and day-6 storage in the room temperature of 25 ± 1 ^{o}C compared to control and E0 (without EtOh in the package). At day 3, the control and E0 treated fruits had been spoilage 90% and 85%, respectively, while the E6, E9 and E12 treated fruits had spoilage 10%, 14% and 15%, respectively. At day 6, 100% of the control fruits and the fruits without EtOH exposure have been spoilage.

At the low temperature of 5 ± 1 ^{o}C and by up 9 day-storage, slow release EtOH in the modified atmosphere packaging significantly reduced the spoilage intensity, the higher of the volume of EtOH released, the lower of spoilage intensity of the fruits. The Figure 1 shows that the spoilage intensity of E0, E3, E6, E9 and E12 were 34%, 30%, 24%, 24%, and 17%, respectively.

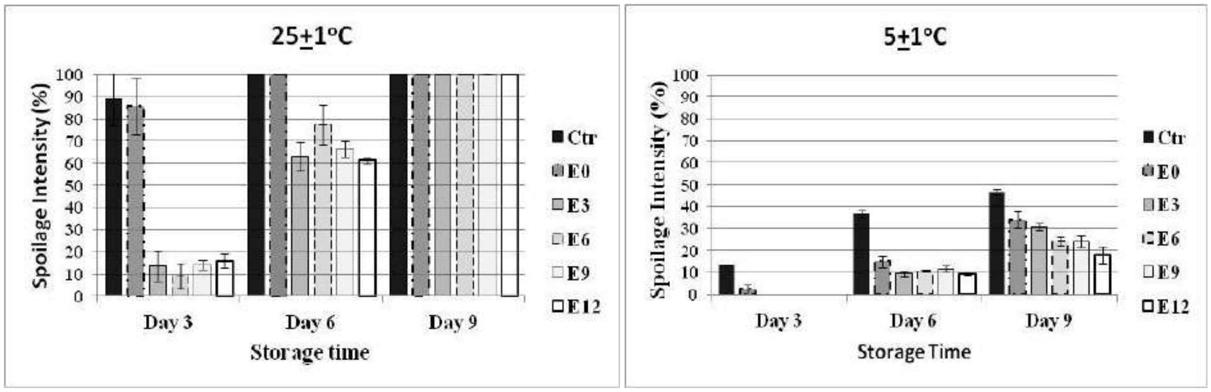

Fig. 1. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the spoilage intensity of strawberry fruits in the modified atmosphere packaging during storage at 25±1 ºC and 5±1 ºC

Almost similar results were shown on the weight loss of the fruits. Slow release EtOH vapor in the modified atmosphere packaging significantly reduced the weight loss of the strawberry fruits by up to day-6 storage at room temperature (25±1 ºC) compared to control and E0 treated fruits of which 100% had been spoilage (Figure 2). While storage by up to 9 days at low temperature (5±1 ºC), slow release of EtOH from E3, E6, E9 and E12 caused significant lower weight loss, namely 0.67%, 0,84%, 0.65% and 0.71%, respectively, compared to control fruits.

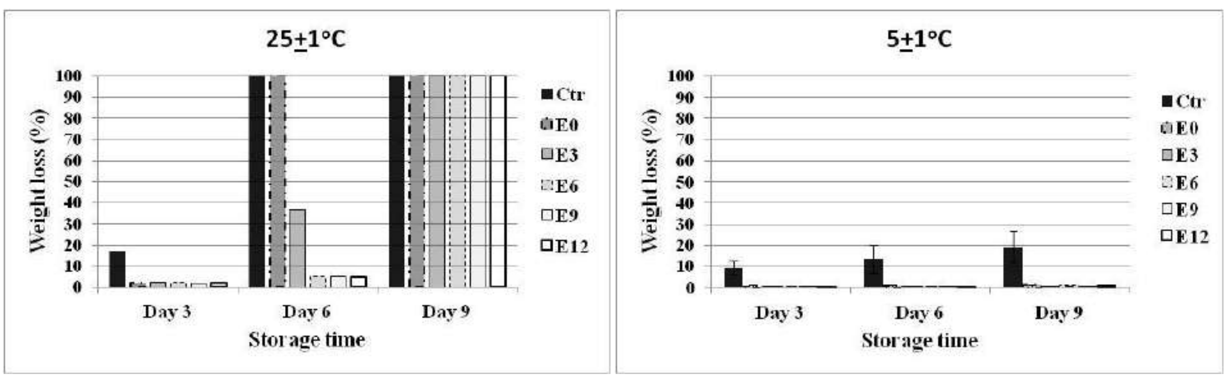

Fig. 2. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the weight loss of strawberry fruits in the modified atmosphere packaging during storage at 25±1 ºC and 5±1 ºC

Based on the analysis above, it is clearly seen that slow release of ethanol vapor and exposed to the strawberry fruits in the modified atmosphere packaging significantly reduced the spoilage intensity and the weight loss of the fruits during storage, both at room temperature of 25±1ºC and low temperature of 5±1ºC. The ability of EtOH vapor in suppressing the growth of decay microorganisms confirms to the report that the vapor was germistatic [19]. The exposure of EtOH vapor on strawberry fruits that was before sliced as cut fresh fruits was reported to be able in maintaining the firmness and reducing the weight loss for one week at 4 ºC [23]. EtOH is a volatile compound and this is important, in particular, to organisms like fungi of which have most vegetative cells exposed to the surrounding atmosphere [27]. The major fungi responsible for postharvest decay of strawberry fruits are *Botrytis cinerea*, followed by *Rhizopus stolonifer, Mucor spp., Colletotrichum spp.,* and *Penicillium spp.* [28].

3.2 Color Differences

There was no interaction between the effects of EtOH vapor and the storage temperature on the color difference between the treated strawberry fruits and the control fruits. At day-3, the slow release of EtOH vapor of E3-E12 at the temperature storage of 25±1°C caused significant color difference between EtOH vapor treated fruits and the control (Figure 3). The control and without treatment of EtOH vapor of the fruits in the modified atmosphere packaging (E0) have been spoilage for about 90% and 85% at day-3 storage at the room temperature of 25±1°C. At day-6, however there was no significant color difference among the EtOH vapor treated fruits in regard to control fruits.

At low temperature of 5±1°C and storage time day-6, the slow release of E9 was optimum to cause color difference of the fruits. While at day-9, the slow release of E12 gave the best color difference of the strawberry fruits referring to the control fruits. This means that the slow release EtOH vapor in the modified atmosphere packaging could be complementary to the low temperature storage in maintaining the color of the fruits. This could occur as EtOH vapor reported to increase the anthocyanin content in the strawberry fruits [23]. The increase of anthocyanin was also reported in strawberry fruits during storage at 7.5°C as resulted of the combine treatments of the EtOH and Methyl Jasmonate vapors [29].

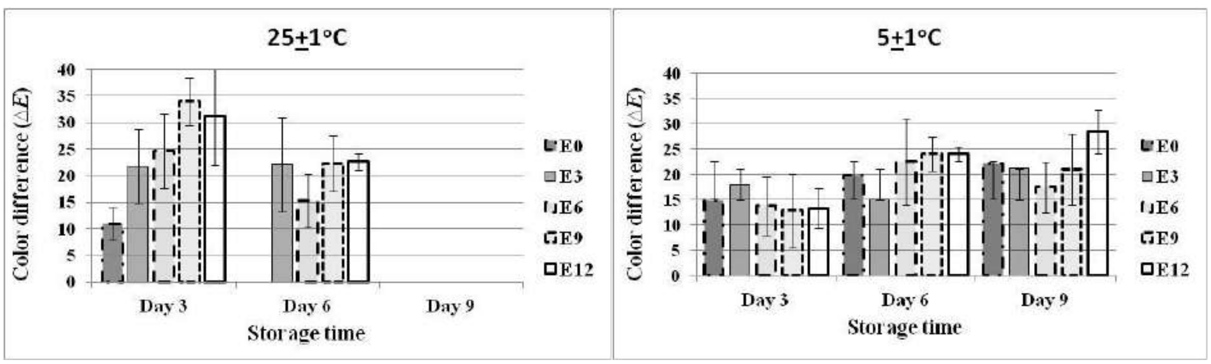

Fig. 3. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the color difference of strawberry fruits in the modified atmosphere packaging during storage at 25±1°C and 5±1°C and control fruits as references.

3.3 Firmness

The temperature of storage and the slow release EtOH vapor independent and significantly affected the firmness of the strawberry fruits in the modified atmosphere packaging. The low temperature of 5±1°C could maintain the firmness of the fruits significantly better than the fruits at room temperature of 25±1°C. The vapor, however, caused higher firmness of the fruits compared to the control and untreated fruits (E0) during storage at 5±1°C. The higher of EtOH given from E3 to E12, the higher of the fruit firmness, as seen at day-6 and day-9 storage on Figure 4. The mode of action of the EtOH vapor in retaining the firmness of the strawberry fruits at low temperature has not been explored. However, a number of studies have found that EtOH can promote and delay the ripening of fruit. Endogenous or exogenous EtOH delayed the ripening of whole fruit, slices and pericarp disks of tomatoes at mature green stage of ripening [13], [14], [15], [16]. The EtOH concentration which delayed the ripening of tomatoes was generally higher than those found naturally in the tissues [16]. Inhibition of ethylene synthesis and carbon dioxide production was suggested to be the mode of action of EtOH in retarding the ripening process [13].

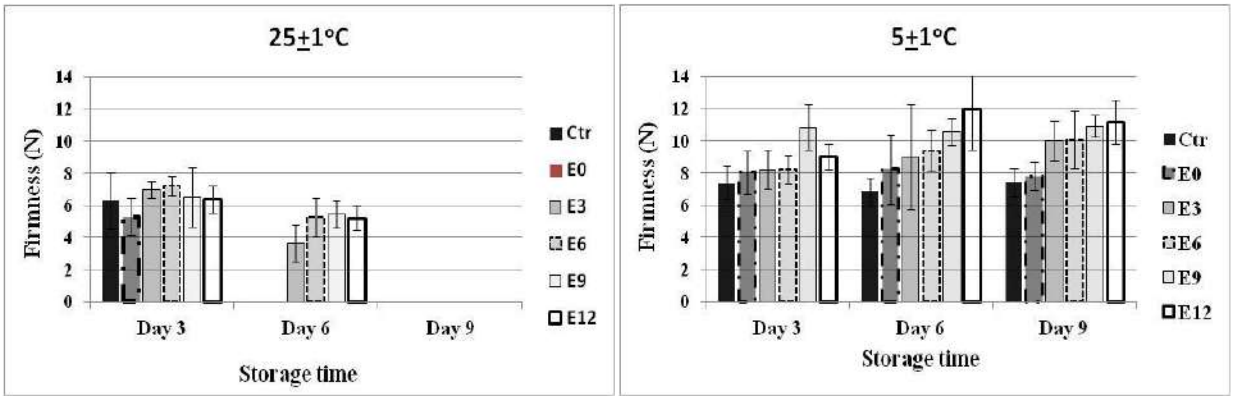

Fig. 4. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the firmness of strawberry fruits in the modified atmosphere packaging during storage at 25±1ºC and 5±1ºC

3.4 Vitamin C and Total Soluble Solids

The effect of storage temperature and slow release of EtOH vapor at day-6 were interact significantly on the Vitamin C content of the strawberry fruits. The increase of EtOH by up to E6 at 25±1ºC, the vitamin C content of the strawberry fruits was higher than the E3 treated fruits (Figure 5). There was no significant difference of the vitamin C content of the fruits when the EtOH was increased to E9 or E12. While storage at 5±1ºC, the treatments of E0-E12 did not give significant different on the vitamin C content of the fruits, also when they were compared to control fruit. At day-9, however, the vitamin C content of the fruits tended to decrease with the higher of EtOH vapor given. The significant decrease of vitamin C occurred on the fruits that were treated with E6, E9 and E12 compared to E0, E3 and control fruits. The decrease of vitamin C is probably due to its degradation by the accumulation of EtOH in flesh of the fruits. The degradation of vitamin C was also found in the wine making fruits due to EtOH [30].

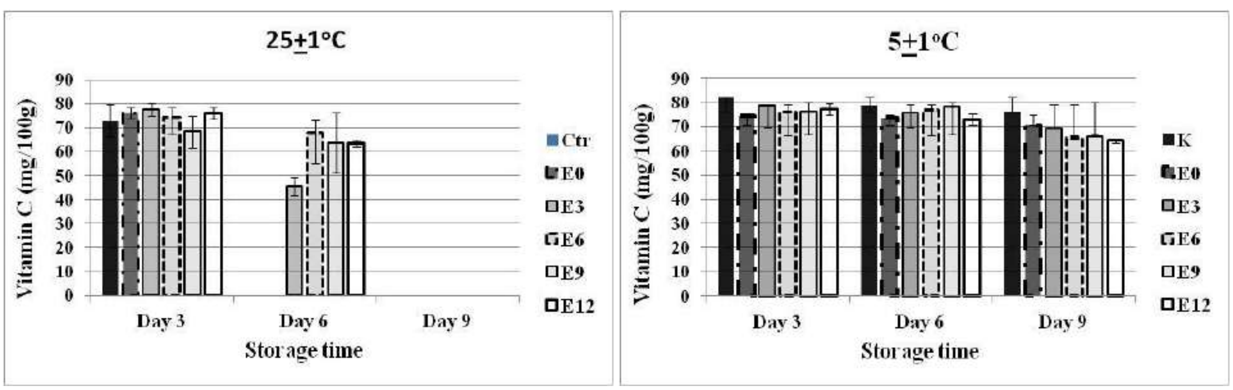

Fig. 5. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the vitamin C content in the flesh of strawberry fruits in the modified atmosphere packaging during storage at 25±1ºC and 5±1ºC

The total soluble solids (TSS) of the fruits was not affected by EtOH vapor which was released in the modified atmosphere packaging when the fruits were stored at low temperature of 5±1ºC.

However, the TSS at day-3 was lower when the modified atmosphere package fruits were stored at room temperature of 25±1ºC (Figure 6). There was no significant different of TSS between the treated EtOH vapor fruits (E3, E6, E9 or E12) and without EtOH vapor treated fruits (E0). This means that the significant difference of the TSS of the control fruits and modified atmosphere package fruits was merely due to the packaging rather than the different volumes of EtOH inside the package. At day-6, however, the TSS of EtOH treated fruits of E6, E9 and E12 were higher than the E3 treated fruits. The lower TSS of the E3 treated fruits was probably due to the growth of fermented microorganisms particularly yeasts.

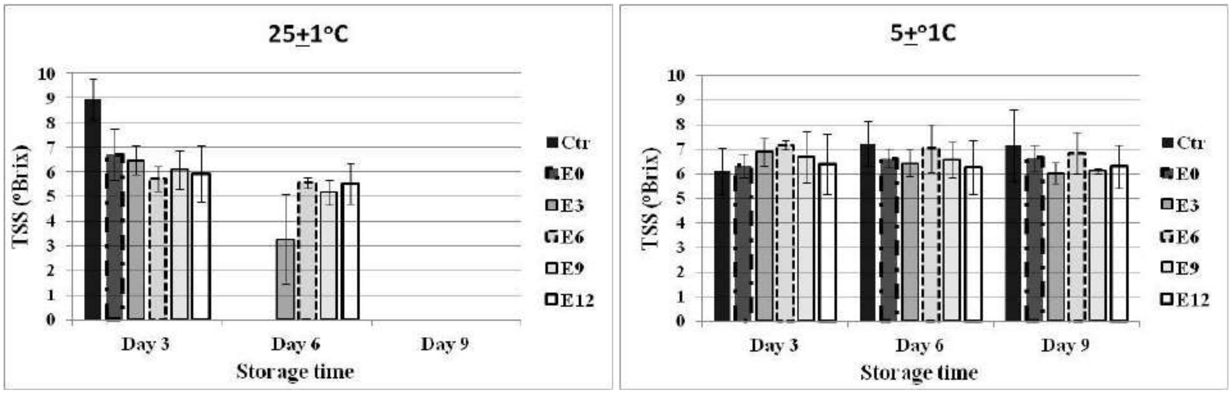

Fig. 6. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the total soluble solids in the flesh of strawberry fruits in the modified atmosphere packaging during storage at 25±1°C and 5±1°C

3.5 Aroma, Color and Taste Preferences

The aroma of the strawberry fruits was affected by the significant interaction between the treatments of the slow release of EtOH in the modified atmosphere packaging and the temperature during storage. At the low temperature of 25±1°C for 3 days, the EtOH vapor released from E9 and E12 caused the lower aroma-preferences of the fruits (neither like nor dislike) compared to the E3 and E6, and control fruits (Figure 6). At day-6, the aroma of E3 treated fruits were still liked, while the E6-E12 treated fruits were neither liked nor disliked.

At low temperature of 5±1°C, the aroma of the fruits was affected by the EtOH vapors. The increase of EtOH vapor, the lower the aroma of the fruits preferred by the panelists. At day-6, however, the aroma preferences were increased to all treated EtOH treated fruits and the control fruits which tended to be very liked by the panelists. While at the day-9, all of the treatments of EtOH vapor and control did not give significant effect on the aroma preferences of the fruits of which tended to be liked by the panelists. The lower scores of the fruits at day-3 storage at 5±1°C were due to the accumulation of EtOH in the flesh of the fruits. The increase of storage time reduced the accumulation of the EtOH in the fruit flesh. This could occur due to higher EtOH vapor pressure in the fruit flesh compared to the pressure in the head space of the modified atmosphere packaging. Therefore, at day-6, the aroma of the overall fruits tended to be very liked by the panelists. At day-9 storage, the aroma of the fruits was still liked by the panelists. This indicated that at the beginning of storage the EtOH vapor pressure was high and caused the diffusion and accumulation of the EtOH in the fruit flesh. Along with the increase of storage time, the EtOH vapor pressure in the fruit flesh become higher compare than the pressure in the headspace of the packaging of which resulted on the movement of the EtOH vapor in the fruit flesh into the headspace. The EtOH is a volatile compound, so that its easily vaporized depended upon the gradient of the vapor pressure between the EtOH in the fruit flesh and the surrounding atmosphere.

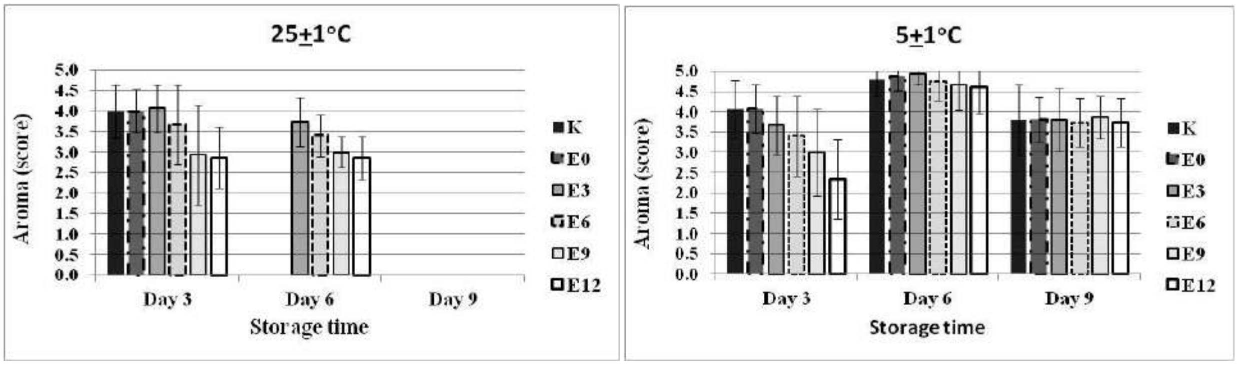

Fig. 7. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the aroma preferences of strawberry fruits in the modified atmosphere packaging during storage at $25\pm1^{o}C$ and $5\pm1^{o}C$

The organoleptic preferences of the strawberry fruit colors were not affected by the slow released of EtOH vapor and the storage temperature, except the fruits that stored at $5\pm1^{o}C$ at day-6, the color of the control fruits was significantly disliked compared to the fruits in the modified atmosphere packaging with or without EtOH which all tended to be liked by the panelists. This means that the retaining of fruit colors was due to the modified atmosphere packaging which resulted in slowing down the respiration rate at low temperature of $5\pm1^{o}C$. However, the strawberry fruits exposed to EtOH vapor caused the elevation of anthocyanin content of the cut fresh fruits during storage for one week at $4^{o}C$ [23].

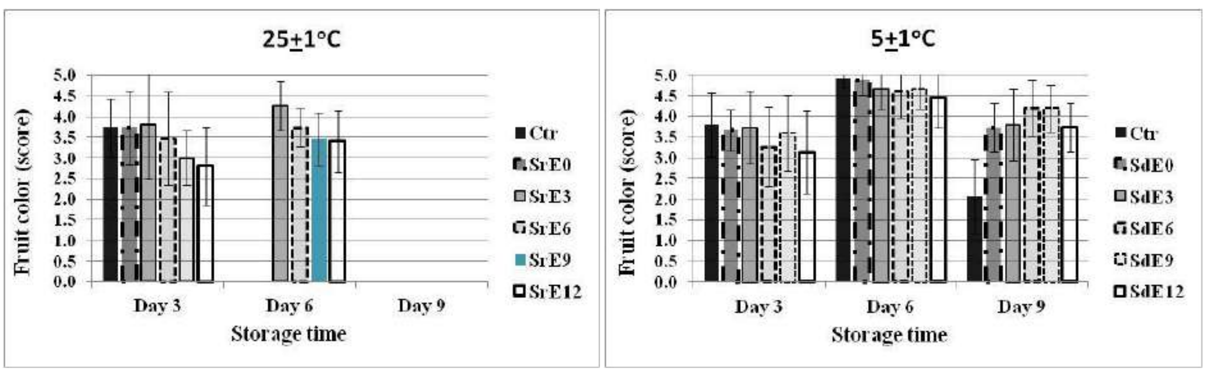

Fig. 8. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the color preferences of strawberry fruits in the modified atmosphere packaging during storage at $25\pm1^{o}C$ and $5\pm1^{o}C$

The taste of the strawberry fruits was merely affected by the slow release EtOH vapor at the day-3 storage. The higher of the volume of EtOH released in the modified atmosphere packaging, the lower preferences of the taste of the fruits were preferred by the panelists. The EtOH vapor treatment of E3, the taste of the fruits tended to be liked by the panelists by up to 6-day storage at room temperature of $25\pm1^{o}C$, and by up to 9-day storage at $5\pm1^{o}C$. While the higher EtOH vapor treatment of E9 and E12, the fruit taste tended to be disliked. Despite the beneficial effects of the EtOH resulted in this experiment such as the reduction of spoilage intensity and weight losses, color differences, firmness, fruit color preferences, and the insignificant effects on Vitamin C, TSS and aroma preferences especially for the fruit stored in modified atmosphere packaging at low temperature of $5\pm1^{o}C$ for longer time by up to 9 days, the taste of the fruits has to be taken consideration. The accumulation of residual EtOH in the flesh fruits as result of the high application of EtOH vapor in the headspace of the packaging is suggested to be the cause of the lower score of the preferred taste. The lowest of EtOH vapor released from E3 in this experiment gave better taste of the fruits compared to the E6-E12 treated fruits. This opens for further experiments by lowering the application rate of the EtOH in the modified atmosphere packaging, or combined with other natural antimicrobial volatiles which are released in the MAP.

Short time exposure of EtOH vapor to the strawberry fruits before they cut to become cut fresh fruit then stored at 4°C could retain the fruit quality during storage [23].

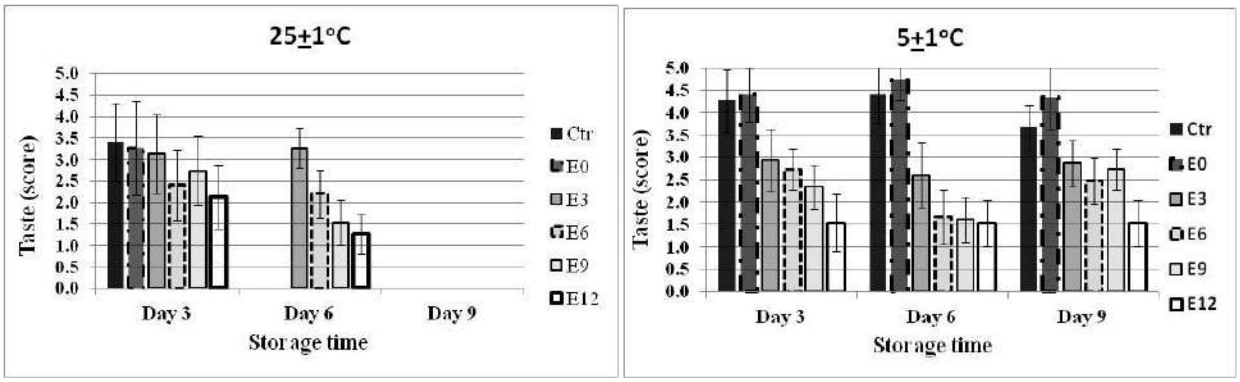

Fig. 9. The effect of slow release of E0 (0 ml), E3 (3 ml), E6 (6 ml), E9 (9 ml) and E12 (12 ml) EtOH on the taste preferences of strawberry fruits in the modified atmosphere packaging during storage at 25±1°C and 5±1°C

4. Conclusion

The slow release of EtOH vapor from different volumes impregnated in the matrixes of carrageen in the MAP significantly reduced the spoilage intensity and the weight loss during storage at 25±1°C and 5±1°C. The EtOH vapor was also able to give better firmness and longer retaining the color of the fruits, as indicated by color differences of the EtOH treated fruits referred to control fruits, compared to the fruits in MAP without EtOH vapor. The slow release of the EtOH vapor was also able to better retaining the TSS and vitamin C content of the strawberry fruits. The sensory evaluation of the fruit aroma and color stored at 25±1°C indicated that the lower EtOH vapor, the better aroma and color of the fruits preferred by the panelists. However, at the low temperature storage of 5±1°C, there was no significant difference of the aroma and color between the EtOH treated and without EtOH treated fruits in the MAP. The sensory evaluation of the taste, however, the taste of EtOH in the fruit was still detected by the panelists. With the high rate of E6, E9 and E12 applied, the taste of the fruits was disliked by the panelists, while the fruits treated by E3 tended to be liked nor disliked.

Despite the fact that EtOH vapor gave beneficial effects on the reduction of spoilage and weight loss, better firmness, longer retaining of the color, TSS, and vitamin C content of the strawberry fruits, and accepted aroma by the panelists, the taste of the fruits, as one of value consumers, has to be taken as important consideration. The high diffusion and easily dissolve in water making EtOH is easily to accumulate in the watery flesh of the strawberry fruit. This opens for further research to apply lower rates of the slow release EtOh in the MAP. With the lower rate, could be interesting to mix the EtOH with an essential volatile oil which has antimicrobial properties, tasteless in the fruit, non-phytotoxic and safe for human.

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Effect of Gum Arabic and Sodium Bicarbonate Coating on Disease Development of Red-fleshed Pitaya Fruit (*Hylocereus polyrhizus*)

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Abstract

Pitaya is a highly perishable fruits that can only last for 5-7 days under room temperature condition. Edible coatings have been used to protect perishable fruit from deterioration by retarding some physiological changes, retain volatile flavour compound as well as reducing microbial growth. In this study, gum Arabic (GA) and sodium bicarbonate (SBC) were used to coat pitaya fruits to evaluate disease development. Firstly, the disease development on fruits coated with 10% GA (w/v), 2% SBC (w/v), 10% GA+2% SBC and control was evaluated by symptomatic observation at 2, 4, 6, and 8 day. Disease incidence and severity was calculated for naturally infected pitaya fruits. Secondly, fungal pathogens on infected fruits were isolated on potato dextrose agar and were identified. To elucidate the infection process on coated pitaya, parameters such as spore germination rate and germ tube length were evaluated. Results showed that coated fruits substantially have reduced development of disease symptoms, decay incidence and severity, germ tube growth and spore germination of *Colletotrichum* sp. which was the causal pathogen of anthracnose disease being identified. However, fruits coated with only 2% SBC were less inhibitory than 10% GA alone as well as 10% GA+2% SBC in controlling disease incidence and severity although the three treatments were significantly different from control treatment.

Pitaya fruits coated with treatments of 10% GA and 10% GA+2% SBC which were artificially inoculated with *Colletotrichum* sp. resulted in a lower rate of disease progression by 50% as compared with the control.

Keywords: anthracnose, microbial growth, mycelium, postharvest treatment, quality

Introduction

Pitaya fruit or commonly known as dragon fruit is one of the fastest growing commodities as well as major export fruits in Vietnam. Besides Vietnam, the commercial pitaya plantings can also be found in Malaysia, Taiwan, and Israel. There are three major species of pitaya planted for commercial, i.e., red-skinned with white flesh, red-skinned with red flesh, and yellow-skinned with white flesh. Unlike Vietnam which is famous for red-skinned with white flesh pitaya, the red-skinned with red flesh or known as red-fleshed pitaya in this study is preferred by Malaysian [1, 2]. This is because Malaysian believed this red-fleshed pitaya is more nutritious than the white-fleshed pitaya. Pitaya is a fast return fruit crop with production in the second year after planting and full production in 5 years [3]. Thus, it gains popularity among fruit growers in Malaysia.

Pitaya is categorized as non-climacteric fruits [4] where it must be harvested at its fully matured stage with peak quality. However, it is a highly perishable fruits that can only last for 5 to 7 days under ambient temperature. Several techniques have been used to extend the holding period for fresh horticultural products. Edible coatings were used to protect perishable food product from deterioration by retarding some physiological changes, retain volatile flavour compound as well as reducing microbial growth [5]. For example, gum Arabic (GA) is found as a good ingredient to be used as coating in mango [6, 7] and papaya [8]. Sodium bicarbonate (SBC) is a compound which is widely used in food industry [9], readily available and cheap. SBC can inhibit *in vitro* mycelia growth of *Bacillus subtilis* in papaya [10, 11].

Therefore, the combinations of GA coating and SBC have been developed for this research purposes. Yet, there is lack of information reported using these coating on pitaya fruit in suppressing disease development during postharvest handling until marketing stage in order to maintain good quality of fruit. Thus, the objectives of this study were i) to evaluate disease development on pitaya fruits coated with GA and SBC, ii) to isolate and identify fungal species on diseased fruit and iii) to determine the initial infection process of the fungal species isolated from infected fruits.

Materials and methods

2.1 Fruit samples preparation

Red-fleshed pitaya fruits (*Hylocereus polyrhizus*) which were free from diseases and blemishes, uniform in size and colour were collected from a pitaya farm located in Sepang, Selangor, Malaysia. These fruits were then washed with distilled water and air-dried before treatment application. The air-dried fruits were then dipped in distilled water (as control), 10% GA solution, 2% SBC solution, and 10% GA+2% SBS solution for 2 min. After 2 min, the fruits were removed from the solution and left to air-dry, followed by placing them carefully in a clean carton box and kept in 26^{o}C room of 95% relative humidity (RH). Each treatment comprised of four replicates with one fruit per replication.

2.2 Disease incidence and severity observation

The fruits were observed every two days for disease incidence using Cubeta *et al.*, [12] and Ding & Mijin [13] methods while disease severity was recorded according to percentage of infected surface area [14].

2.3 Isolation and identification of fungal genera on diseased pitaya fruits

Tissues of diseased fruits was isolated and cultured on potato dextrose agar (PDA). The plates were incubated at room temperature of 25-27°C for 3 to 7 days and examined daily for sporulation under low-power stereoscopic microscope [15]. A pure culture of possible causal pathogens was carried out by sub-culturing. Identification was carried out by evaluating the characteristics of symptoms visually and the morphology of fungal pathogen microscopically using light microscope.

2.4 Determination of initial infection process of the fungal species isolated from infected pitaya fruits

After isolation and identification of the causal pathogen of pitaya fruit through the examination of suspected pathogen macroscopically and microscopically, pure culture which had been incubated for 5 to 7 days was used as inoculums for *in vivo* test. Disease and defect free fruits were obtained from the same farm as above. The fruits were cleaned and rinsed with sterilized distilled water followed by air dried. Fruits were then coated with the four treatments as mentioned above. After coating the fruits were allowed to air dry for 24h. Then each pitaya fruit was surface inoculated by swabbing with spore suspension of the pathogen using cheesecloth at marked spots.

Fruits for control were inoculated with sterilized distilled water. Fruits were then placed in four clean carton boxes according to treatments and incubated at 26°C room with 95% RH for 24h.

Each treatment comprised of three replicates with one fruit per treatment.

For spore germination rate, a fruit from each treatment was used for evaluation. A total of three samples were taken randomly from a fruit to observe for germinated spore after 24h incubation at 26°C [16]. The observations were performed at four time points (0, 2, 4, and 6 h after inoculation) using at least 100 spores per replicate and calculated according to Droby *et al*., [17]. For germ tube length measurement, it was measured at the same time germination rate data collection by using an ocular micrometre.

2.5 Experimental design and statistical Analysis

The experiment was arranged in a completely randomized design (CRD). The obtained data was subjected to Analysis of Variance (ANOVA) and mean separations were performed using the least significant difference (LSD) test (P≤0.05). Statistical Analysis System (SAS) was used to perform the analysis. Data for percentage of disease severity and germinated spores were transformed into the arc sine square root values to normalize distribution before ANOVA analysis.

The percentages shown are untransformed data.

Results and discussion

3.1 Evaluation on disease development of pitaya fruits coated with GA and SBC

The symptoms of disease infection started on day 4 with control fruit showed sunken yellowish-brown spots on the fruit surfaces while GA and SBC coated fruits were still in good visual quality.

By day 6, the sunken yellowish-brown spots on control fruits enlarged while other treated fruits started to have similar symptoms as in control fruits but less severe. However, fruits of 10% GA coating and 10% GA+2% SBC had smaller sunken spots as compared to fruits treated with 2% SBC. By day 8, fruits of control and 2% SBC were covered with black spores, soft, rotten and with unpleasant smell. At the same time, although the fruits treated with 10% GA and 10% GA+2% SBC also showed disease symptoms, the symptom was less severe than control and 2% SBC.

Fruits coated with GA and SBC both virtually delayed disease symptom development as compared to control. According to Aharoni *et al*., [18], SBC at a concentration of 2% has potential in controlling fruits fungal decay during prolonged storage and shelf life, while maintaining fruit quality. However, the results of this study did not agree with them as pitaya fruits treated with 2% SBC had higher infection rate than fruits treated with 10% GA and 10% GA+2% SBC at the later stage of storage days. The pitaya fruits coated with GA+SBC have better result than the SBC alone. This could probably due to GA which acts as physical barrier that prevent disease transmission as reported by Gamagae *et al*., [19].

3.2 Disease incidence and disease severity

There was no significant interaction between coating and day on disease incidence of pitaya fruit but not for disease severity (Table 1). The pitaya fruits coated with 10% GA+2% SBC, 10% GA and 2% SBC showed significantly (P≤0.05) lower disease incidence and disease severity than the control fruits. There were no significant differences in pitaya fruits treated with 10% GA, 10% GA+2% SBC, and 2% SBC. Since the severity evaluation has better accuracy, the results of disease severity showed that 2% SBC was less effective in controlling fruit decay and the lesion was greater than those of GA coating alone (Table 1). However, the 10% GA+2% SBC coating did not show difference in disease severity with GA coating alone.

In this experiment, disease incidence and severity caused by fungal pathogen were significantly reduced in pitaya fruits treated with SBC as compared to control. This result was in agreement with observation done and proven by Karabulut *et al.*, [20] where SBC was effective in controlling the decay of sweet cherry. However, 2% SBC was not effective in reducing pitaya disease severity as compared to fruits coated only with 10% GA and combination of both. This phenomenon may be related to species specificity of fungal pathogens which have different sensitivities to SBC [21].

Both the disease incidence and severity of treated and untreated pitaya fruits increased significantly as the storage days progressed (Table 1). This is usually associated with ripening changes in the physiological condition of the fruit tissue [22] especially infected fruits act as food sources to the secondary microorganisms.

Table 1. Effects of gum Arabic coating treatments with or without 2% sodium bicarbonate incorporation on disease incidence and severity of red-fleshed pitaya fruits stored for eight days at room temperature of 26°C/95 relative humidity

Factor	Disease incidence (%)	Disease severity (%)
Treatments (T)		
Control	68.8 a^{z}	51.33 a
10% GA	37.5 b	16.8 c
10% GA+2% SBC	37.5 b	17.7 c
2% SBC	37.5 b	42.3 b
Days after treatment (D)		
2	0 c	0 c
4	12.5 c	11 c
6	62.5 b	21.9 b
8	100 a	43.6 a
Interaction		
T x D	NS	*

^{z}Each value is a mean of four replicates; values followed by different letters in a column are significantly different ($P\leq0.05$) according to LSD test NS,
** Non-significant or significant at $P\leq0.05$*

3.3 Isolation and identification of fungal genera on pitaya fruits showing disease symptoms

Disease symptom was clearly observed on fruit from day 4 onwards during the storage and the symptoms were described as spots of circular, sunken lesions, brownish in colour with some black spores on the infected areas (Fig. 1A). Besides, fruit tissues at the infected areas were soft, enlarged, rotten, dead and gradually infected by secondary pathogen or saprophytes as storage days progressed (Fig. 1B). When the isolated infected pitaya fruit skin was observed using a compound microscope under the magnification of 30x, some fruiting bodies resembled acervuline were found (Figs. 2A and B). The bodies were black in colour, needle-like structure that penetrated through the cuticle of fruit skin.

After a series of isolation work, a total of 21 plates of pure cultures were isolated from the infected fruit skins and were categorized into four different types of fungi according to their characteristics. Through visual observation on the first culture obtained, it appeared as pinkish to orangey with white mycelium ring-pattern (Fig. 3A). The spores varied in size with three to five cells in curved end-shaped (Fig. 3B). The second type of culture obtained was black in colour due to black mycelium formed with slight grayish circle-like pattern (Fig. 4A). The spores were large with rounded ends and comprised of four to seven cells (Fig. 4B). For the third type of culture, it appeared as white mycelium with orange and black rings patterned alternately (Fig. 5A). The spores were cylindrical in shape with one cell (Fig. 5B). The mycelium of fourth type culture showed a combination of various colours such as grayish green, brown and white colour with irregular edges (Fig. 6A). This type of culture failed to produce spores but only with clumps of hyphae although after incubated for 7 days (Fig. 6B).

Fig. 1. (A) Disease infected pitaya fruits showed spots of circular, sunken lesions, brownish in colour with black spores. (B) A fruit with infection by secondary pathogen or saprophytes

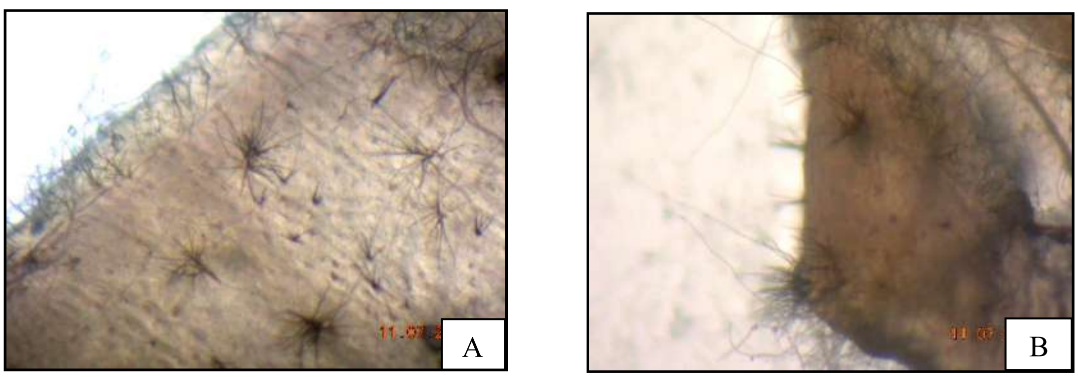

Fig. 2. (A) A front view of acervuli found on the surface of infected pitaya fruit skin. (B) The side view of pitaya fruit skin acervuli

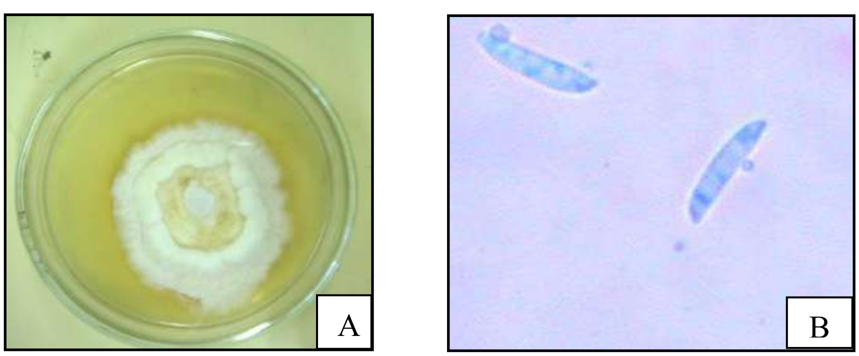

Fig. 3. The first type of fungi obtained after a series of isolation. (A) Culture of pinkish to orangey with white mycelium ring-pattern. (B) Spores with three to five cells in curved end-shaped. x400

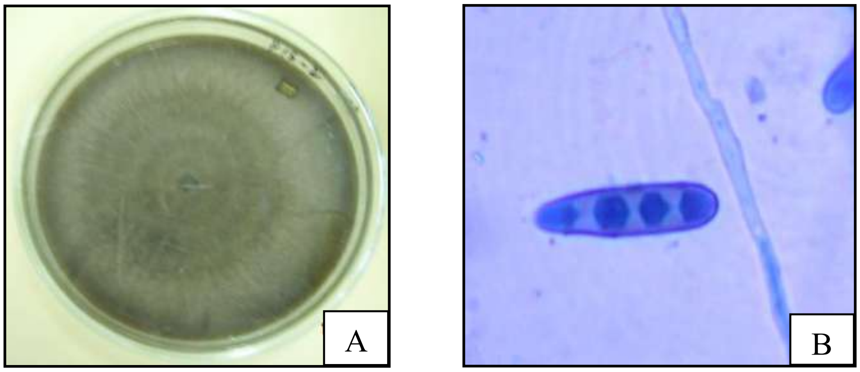

Fig. 4. The second type of fungi obtained after a series of isolation. (A) Culture of black mycelium formed with slight grayish circle-like pattern. (B) A large rounded ends spore with four to seven celled. x400

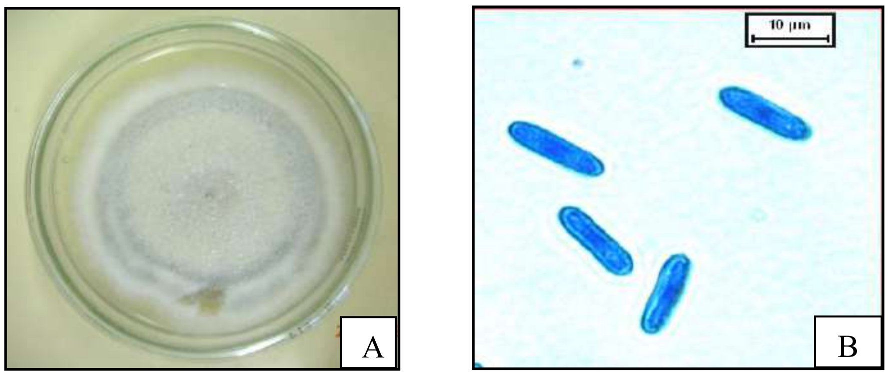

Fig. 5. Third type of fungi obtained after a series of isolation. (A) White mycelium with orange and black rings patterns alternately found in the culture. (B) Spores with one celled and were cylindrical in shapes

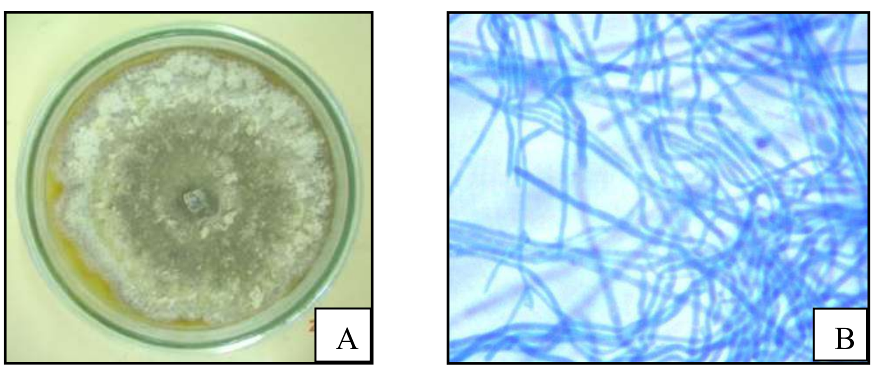

Fig. 6. The fourth type of fungi obtained after a series of isolation. (A) Mycelium in combination of various colours such as grayish green, brown and white colour with irregular edges. (B) The culture failed to produce spores but only with clumps of hyphae. x400

After underwent a series of symptomatic and microscopic observations, cultures were all identified accordingly and the results showed that the first type of culture was *Fusarium* spp.

According to keys by Watanabe [23], the morphological characteristics of *Fusarium* spp. are described as hyaline conidiophores, macroconidia with slightly curved apical cells, usually three to five celled and the colonies on PDA are pale yellowish brown or pinkish brown in colour. While the second type was found to be *Drechslera* spp., which have pale brown conidiophores, conidia are cylindrical, straight or curved partially, mainly six to seven celled [23]. The third type of culture was *Colletotrichum* spp. This fungus produces conidia-bearing acervuli. It produces colourless, one celled, ovoid, cylindrical conidia. The masses of conidia appear pink or salmon colour [18]. While the last type was believed to be saprophytes as they did not produce spores during the incubation period. According to Agrios [24], saprophytes are grown on dead, injured or processed plant products; it builds up large masses of mycelium.

The possible causal pathogen on diseased pitaya fruit was successfully identified as *Colletotrichum* spp. that causes anthracnose disease. This was proven by the symptoms developed on the naturally infected pitaya fruits which corresponded to symptoms that caused by *Colletotrichum* spp. The symptoms were small circular, watery, dark, brownish-black sunken lesion spots resembling indentations caused by blunt circular objects; spots enlarge as fruit soften and central part becomes dark due to development of black acervuli beneath the skin [24].

The appearance of acervuli which observed on the infected pitaya fruit skin strengthens the results of identification. According to Agrios [24], *Colletotrichum* is an acervulus-producing mitosporic fungi. Acervuli are sub-epidermal structures and break out through the surface of the plant tissue as found in Figs 2A and B in this study. As such, *Colletotrichum* spp. was artificially inoculated on red-fleshed pitaya fruit that were treated as above. Following the inoculation, the *Colletotrichum* spp. spore germination rate and germ tube length was measured.

3.4 Spore germination rate

Percentage of spore germination of *Colletotrichum* spp. on artificially inoculated pitaya fruits which were treated with 10% GA, 2% SBC and 10% GA+2% SBC was significantly ($P \leq 0.05$) lower than control (Table 2). As time progressed, the spore germination rate of *Colletotrichum* spp. increased. However, there were no significant differences among 2, 4 and 6h.

The inhibitory effect of SBC on postharvest pathogens is most likely due to the reduction of fungal cell turgor pressure brings to collapse and shrinkage of hyphae and spores and consequent sporulation inability [25]. This was also proven by the previous findings by which SBC was found to inhibit *in vitro* mycelia growth of *Alternaria alternata, Fusarium* spp*., Ralstonia stolonifer* [26].

After 24h of incubation of artificially inoculated pitaya fruit, the first two hours did not show any difference in spore germination rate among treatments, indicating spores of *Colletotrichum* spp. only started to germinate after 4h of inoculation. Gradually, more spores germinated at 4 and 6h as shown in Table 2.

Table 2. Effects of gum Arabic coating treatments with and without 2% sodium bicarbonate incorporation on spore germination rate of *Colletotrichum* spp. on pitaya fruits at room temperature of 26°C after 24 h incubation

Factor	Spore germination (%)
Treatments (T)	
Control	16.8 a[z]
10% GA	10.5 b
10% GA+2% SBC	9.6 b
2% SBC	10.1 b
Time point (h)	
0	7.1 b
2	11.1 ab
4	13.3 a
6	15.4 a
Interaction	
T x h	NS

[z]Each value is a mean of three replicates; values followed by different letters in a column are significantly different ($P \leq 0.05$) according to LSD test.
NS Non-significant at $P \leq 0.05$.

3.5 Germ tube length

The results of this study showed that the germ tube growth of *Colletotrichum* spp. has been successfully retarded in fruits that treated with 10% GA or 10% GA+2% SBC as compared to control and 2% SBC (Table 3). Two h after incubation, fruits coated with 10% GA+2% SBC showed the shortest germ tube length. While fruits treated with 10% GA showed the shortest germ tube length among all treatments after 4 and 6 h of inoculation. Surprisingly, at the time point of 2 and 4 h, pitaya fruits treated with 2% SBC was ineffective in reducing germ tube growth of the pathogen when it is expected to have better effect compared to control fruits.

In this experiment, fruits treated with GA as coating showed most effective in controlling germ tube growth of the pathogen. The strong impact of GA as coating in suppressing disease symptoms development made clear that it did have a role in controlling pitaya fruit disease. The possible explanation of this effect is the thick layer of GA coat has reduced internal oxygen levels which gave a direct impact on fungal metabolism, affecting its ability to invade the fruit tissues [26].

Usually germ tube elongation requires conditions that favour them to grow and multiply [24].

Table 3. Effects of gum Arabic (GA) and sodium bicarbonate (SBS) coating on germ tube elongation of *Colletotrichum* spp. on pitaya fruits incubated at 26°C/95% relative humidity for 24h

	Germ tube length (μm ± SD)			
Time point (h)	Control	10% GA	10% GA + 2% SBC	2% SBC
0	10.90^z±4.61^y	15.15±1.39	16.92±4.12	17.60±7.98
2	17.31±0.95	16.01±4.88	13.91±0.41	18.23±5.15
4	17.92±4.01	14.82±3.46	17.48±4.01	21.72±2.42
6	23.43±5.49	12.53±2.26	16.19±1.47	14.86±3.15

^{z}Each value is a mean of 66 germ tubes lengths measured.
^{y}Data represented standard deviations of the means.

4. Conclusion

The findings of this study suggested that the combination of 10% GA with 2% SBC is the best treatment to protect pitaya fruits from postharvest disease infection. However, further study should focus on testing the efficacy of combined application of wax coating and SBC under commercial practices as well as combination with other compounds. These compounds can either be inorganic or organic such as sodium chlorite, potassium bicarbonate, ammonium bicarbonate and chitosan as additives, so as to obtain even better control.

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EMERGING TRENDS OF HORTICULTURE IMPACTING GLOBAL MARKETS: THE SOCIO-ECONOMIC STUDIES

Adoption Level of Recommended Technology for Disease-free Citrus Seedling Production

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Abstract

The support of a robust seedling industry is very much important to develop citrus agribusiness that is competitive and sustainable. The provision of the certified citrus seedling is one of the determinants of the success of citrus farming. The production technology of disease-free certified citrus seedling has been socialized and disseminated to citrus seedling producers in many citrus seedling production zones in Indonesia, among them is in Sambas Regency, West Kalimantan and Purworejo Regency, Central Java. Citrus seedling production should follow the model of recommended technology of blue-labelled citrus seedling production in polybag. The objective of this study was to identify the adoption level of the production technology of disease-free citrus seedling, in order to provide feedback for the capacity improvement of certified citrus seedling producers. The study was conducted in Sambas Regency, West Kalimantan and Purworejo Regency, Central Java. Data collection was by a survey towards respondents of certified citrus seedling producers. Data were analyzed by descriptive statistics. The study results indicated that the adoption level of most technology components in Purworejo Regency was relatively higher than Sambas Regency. Further efforts in improving the capacity of citrus seedling producers in an effective way are necessary to be done.

Keywords: Adoption, technology, citrus, rootstock, seedling

Introduction

The seedling industry is a part of the upstream sector in citrus agribusiness in Indonesia. The support of a robust seedling industry is very much important to develop the citrus agribusiness that is competitive and sustainable [1]. The provision of citrus seedling with good quality is one of the determinants of the success of citrus farming. This means that the use of high-quality seedling could sustain the farming, on assumption that the use of other production inputs, such as fertilizer, pesticides, irrigation and labour, are optimum.

A good-quality citrus seedling is a disease-free certified citrus seedling that has specifications of: (1) free of systemic pathogens, i.e. CVPD (Citrus Vein Phloem Degeneration), CTV (Citrus Tristeza Virus), CVEV (Citrus Vein Enation Virus), CEV (Citrus Exocortis Viroid) and CPsV (Citrus Psorosis Virus); (2) the purity of the scion and rootstock is guaranteed; (3) the stages of the production process follow the regulations of seedling monitoring and certification [2]. The use of disease-free certified citrus seedling is highly important to diminish the attack risk of CVPD disease resulting in the decrease of citrus productivity and quality, also causing the death of the citrus crops. A study case in Sambas Regency showed that CVPD has infected 31% citrus crops in this area, causing the crops to death and must be eradicated [3].

Certification procedure of citrus seedling has been regulated in the Decree of Agriculture Minister of the Republic of Indonesia No. 201/Kpts/SR.130/D/11/2016 about certification technique of horticulture seedling. Furthermore, the citrus seedling production process should be in accordance with the model of recommended technology of disease-free citrus seedling production that has been socialized and disseminated by Indonesian Agency for Agricultural Research and Development (IAARD) through Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI). This production technology model refers to the technology recommendation for certified (blue-labelled) disease-free citrus seedling production in polybag, i.e., (1) using polybag in the whole production process, (2) sowing rootstock seed correctly, (3) selection of nucellar seedling, (4) using bud-stick from Bud-stick Multiplication Block, (5) grafting area is minimum 20 cm above root crown, and (6) optimum maintenance during production process [1].

The production technology of disease-free certified citrus seedling has been socialized and disseminated to citrus seedling producers in many citrus seedling production zones in Indonesia.

The citrus seedling producers have been fostered through a training and technical guidance on recommended technology of disease-free citrus seedling production. Therefore, further adaptive assessment is necessary to measure the implementation of this technology and to obtain information on the level of technology adoption by citrus seedling producers.

Technology adoption is a mental process or behaviour change, both in the form of knowledge (cognitive), attitude (affective), and skill (psychomotor) in a person since he knows innovation until decided to adopt it after receiving innovation [4]. Adoption of innovation by adopters will occur after going through the mental process. The process starts from attention, then interest will grow, and subsequently, a desire arises to try the innovation. These processes are encouraging adopter to make decisions and finally arrive at efforts to encourage the implementation of technology as an action called adoption [4]. Several factors influencing agricultural technology adoption include socio-economic conditions, such as the adopter's education, farming experience, technology assistance and the distance between the technology source and farmer location [5]. In addition, the support of agricultural extension and the availability of technology information and communication are also important factors for technology adoption [6].

The objective of this study was to identify the adoption level of the production technology of disease-free citrus seedling among citrus seedling producers, in order to provide feedback for the capacity improvement of certified citrus seedling producers. This article complements the extant literature by assessing the technology implementation and adoption of disease-free citrus seedling production which has not been discussed in any previous literature.

Methodology

The study was conducted in the citrus seedling production area of Sambas Regency, West Kalimantan and Purworejo Regency, Central Java on October to November 2017. Data collection was done by survey method towards thirty key respondents, in total, of certified citrus seedling producers of both locations. These respondents represented a combination of nine seedling producer groups and two private producers over Sambas Regency and Purworejo Regency. One seedling producer group usually has 5-8 members, on average. Head of the farmer group had a Certificate of Seedling Producer issued by the local government that enables him to produce certified/blue-labelled citrus seedlings, while the members became his work partners and followed what was recommended by the group leader based on the valid regulations in producing citrus seedlings [1]. Thus, each producer group typically performed a uniform producing method. All seedlings meeting the product specifications were subsequently labelled by the local Seed Monitoring and Certification Institute.

A questionnaire was developed to collect data on the adoption of the component of the technology recommendation for certified (blue-labelled) citrus seedling production in the polybag.

Afterwards, data generated were analysed and interpreted descriptively. In the data analysis process, results were presented in contingency tables, and the determination of technology adoption level utilized a percentage tabulation that refers to the method of previous study regarding technology adoption level [7], in which the adoption rate was divided into four categories as follows (Table 1).

Table 1. Categories of the adoption level of the technology recommendation for certified (blue-labelled) disease-free citrus seedling production in polybag

Percentage	Category of adoption level
>75%	Very high
51-75%	High
25-50%	Low
<25%	Very low

Results and Discussion

Demographic characteristics of the sample of citrus seedling producers

In Sambas regency, West Kalimantan, the majority (75%) of certified citrus seedling producers made the seed nursery activities as their primary occupation, and the remaining (25%) had another primary job so that seed nursery activity was just a secondary work. This characteristic may influence the time allocation for seed nursery activities. The majority (72.22%) of the sample had experience as seed producer for more than 10 years, while others (27.78%) had been in this seed industry for less than 10 years. The sample was mainly 31-40 years of age (44.4%) and 41-50 years of age (33.3%). In terms of educational background, the majority (50% in total) of certified citrus seed producers had a high school education (junior high and senior high). Only a few respondents (15%) had graduate and postgraduate education.

Meanwhile, in Purworejo, all (100%) certified citrus seedling producers within the sample made seed nursery activities as the main occupation. In terms of experience, the majority (70%) of the sample had experience as seed producer for more than 10 years, while others (30%) had been in the industry for less than 10 years. The sample was primarily 31-40 years of age (60%), thus citrus seed producers in Purworejo was relatively younger than Sambas. For education, the majority (44.4%) of the sample had a senior high school level, while others (33.3%) had an elementary school education. Higher education is quite important in playing the role in certified citrus nursery activities because they were able to understand the importance of the disease-free certified citrus seedling.

Adoption level of technology recommendation for disease-free citrus seedling production

Using polybag during the whole production process

The production technology of disease-free citrus seedling has recommended using polybag in the whole production process, from the stage of sowing rootstock seeds to the stage of being ready for sale. However, the adoption of this particular recommendation was still very low, as none of the citrus seed producers of both districts (Sambas and Purworejo) implemented this method (Table 2). They usually carry out the production process on raised beds first, then transfer the successfully grafted planting materials into the polybags. Thus, the production process was not completely done in polybags.

Citrus producers in Sambas sowed rootstock seeds either on raised beds (90%) or trays (10%), and those in Purworejo fully sowed rootstock seeds on raised beds. The importance of using polybags during the whole production process is to maintain the quality growth of the roots system. Meanwhile, in terms of the use of seeding media, citrus seed producers in Sambas mostly used topsoil from latosol + husk charcoal, while those in Purworejo often used topsoil from latosol + husk charcoal or sandy soil + manure.

Table 2. Adoption level of recommended technology for disease-free citrus seedling production

	Technology recommendation of disease-free citrus seedling production	**Sambas, West Kalimantan**			**Purworejo, Central Java**		
		Apply	**Do Not Apply**	**Level of adoption**	**Apply**	**Do Not Apply**	**Level of adoption**
1	**Using polybags in the whole production process**	0	100	Very low	0	100	Very low
2	**Sowing rootstock seed correctly**						
	Placing the pointed part of the seed at the bottom	40	60	Low	100	0	Very high
	Planting the rootstock seeds uses spacing	45	55	Low	100	0	Very high
	Shelter with plastic or other materials	5	95	Very low	100	0	Very high
	Covering the seeding with paranet	60	40	High	88,9	11,1	Very high
3	**Roughing, nucellar selection**						
	Remove rootstock seedlings having yellow leaves	100	0	Very high	88,9	11,1	Very high
	Remove rootstock seedlings performing bent roots	90	10	Very high	55,6	44,4	High
	Remove rootstock seedlings performing stunted growth	75	25	High	55,6	44,4	High
	Remove rootstock seedlings performing extremely fast growth	20	80	Very low	55,6	44,4	High
	Remove rootstock seedlings performing leaf shape changes (turn into rounded, long and pointed, trifoliate) or different with the normal JC/RL leaf shape	80	20	Very high	100	0	Very high
	Remove rootstock seedlings having violet young leaves or different with the normal colour of JC/RL	95	5	Very high	100	0	Very high
4	**Transplanting**						
	Transplanting at 2.5 – 3 months after rootstock seed germination	65	35	High	100	0	Very high
	Cutting half portion of the leaves	10	90	Very low	77,8	22,2	Very high
	Dipping the roots into a mix of mud and fungicide	80	20	Very high	66,7	33,3	High
5	**Using bud-stick from Bud-stick Multiplication Block**	100	0	Very high	100	0	Very high
6	**Grafting**						
	Grafting at area of 20 – 25 cm above root crown	100	0	Very high	100	0	Very high

Technology recommendation of disease-free citrus seedling production		Sambas, West Kalimantan			Purworejo, Central Java		
		Apply	Do Not Apply	Level of adoption	Apply	Do Not Apply	Level of adoption
	Binding the grafting rope moves from the bottom upwards	90	10	Very high	100	0	Very high
	Placing the grafted seedling under a roofed space or covered with plastic	40	60	Low	66,7	33,3	High
7	**Optimum maintenance**	100	0	Very high	100	0	Very high

Sowing rootstock seed correctly

Sowing rootstock seed correctly reflects the correct methods to plant the citrus seed rootstock to prevent bent roots. It is recommended to sow rootstock seeds into nursery polybags with a diameter of 30-40 cm. However, neither producers in Sambas and Purworejo employed this method.

Furthermore, the recommendation for sowing rootstock seed correctly includes placing the pointed part of the seed at the bottom, planting the rootstock seeds uses spacing, sheltering with plastic or other materials, and covering the seeding with gauze or similar material so that the watering does not shift the position of the seeds which can result in bent roots.

Not many citrus seedling producers in Sambas Regency placed the pointed part of the seed at the bottom and not all of them planted the rootstock seeds by spacing because they merely spread the seeds on the raised beds. Thus, the adoption level for this recommendation was low. On the contrary, seedling producers in Purworejo Regency had implemented the placement of the pointed part of the seed at the bottom and planting the rootstock seeds with spacing. Therefore, the adoption level for this recommendation was very high (Table 2).

Roughing/Selection of nucellar seedling

Performing selection for a nucellar seedling is also one important stage to produce successful high-quality citrus seedlings. Only nucellar seedlings of rootstock will be used in the next production process, while generative seedlings should be removed. Therefore, rootstock seedlings having yellow leaves, bent roots, stunted performance, extremely fast growth, leaf shape changes (turn into rounded, long and pointed, trifoliate, or different with the normal JC/RL leaf shape), and violet (or different with the normal colour of JC/RL) young leaves must be discarded.

In Sambas Regency, most citrus seedling producers had implemented almost all of the component of roughing, except discarding rootstock seedlings that perform extremely fast growth.

Therefore, the adoption level of each component of this selection of nucellar seedling was in the category of high to very high, except for discarding rootstock seedlings performing extremely fast growth was in the category of very low. This was because producers in Sambas considered those having very fast growth were still in a good performance, so they seemed reluctant to discard those seedlings, even though this act was actually incorrect. On the contrary, certified seedling producers in Purworejo Regency had applied all aspects of the selection of nucellar seedlings, and thereby the adoption level of each component was in the category of high to very high (Table 2).

A previous related study in TTS district of East Nusa Tenggara showed that selection of nucellar seedling of rootstock was one technology component that was adopted slowly by citrus seedling producers because specific knowledge is required to identify nucellar/vegetative seedling. Moreover, citrus seed producers were often not aware of this matter [1].

Transplanting

Transplanting is a process to move straight-rooted nucellar seedlings from nursery polybags into individual polybags filled with planting media and is maintained optimally until the rootstock is ready for grafting. It is recommended that transplanting is conducted at 2.5-3 months after rootstock seed germination. Transplanting only straight-rooted nucellar seedling will guarantee its quality and the purity of variety. Other important components in transplanting are cutting approximately half portion of the leaves, and dipping the roots into a mix of mud and fungicide in order to coat the roots of nucellar seedlings to avoid excessive evaporation during the transplanting process.

In Sambas Regency, the adoption level of transplanting at 2.5-3 months after rootstock seed germination was high, whereas that of cutting half portion of the leaves was very low, and that of dipping the roots into a mix of mud and fungicide was very high. Thus, not all components of the recommendation for transplanting had been applied by the seedling producers. As for Purworejo Regency, the adoption level of transplanting at 2.5-3 months after rootstock seed germination was very high, that of cutting half portion of the leaves was very high, and that of dipping the roots into a mix of mud and fungicide was high (Table 2). Thus, most producers in Purworejo had applied all aspects recommended for transplanting.

Using bud-stick from Bud-stick Multiplication Block

Bud-stick Multiplication Block is an insect-proof and double door screen house for citrus mother plants grown on raised beds by a certain planting space. These mother plants are grafted from Foundation Block and have the function to generate bud-stick branches used for the citrus nursery. The use of bud-stick from Bud-stick Multiplication Block for grafting stage is an absolute requirement in order to produce disease-free citrus seedlings. All certified citrus seedling producers in both locations had implemented this recommendation, and thereby the adoption level was very high (Table 2).

Grafting

It is recommended to conduct grafting at the stem area of 20-25 cm above root crown when the rootstock seedling has reached ≥50 cm of height. Subsequently, it is also recommended to bind the grafting rope moves from the bottom upwards in order to prevent water from entering inside the grafting linkage. Next, the recommendation to place the grafted seedling under a roofed space or covered with plastic.

The adoption level of grafting at the area of 20-25 cm above root crown was very high, as all producers in both locations had adopted this recommendation (Table 2). Most producers in Sambas and Purworejo started to perform grafting when the rootstock seedling has reached ≥50 cm of height, while others started conducting the grafting when the rootstock seedling has reached ≥40 cm to <50 cm. In terms of binding the grafting rope moves from the bottom upwards, the adoption level of this method in both districts was also very high, because most producers had implemented this method. However, the recommendation to place the grafted seedling under a roofed space or covered with plastic was still adopted low in Sambas Regency, whereas in Purworejo Regency, this method was adopted highly (Table 2).

Optimum maintenance during production process

Optimum maintenance for rootstock seedlings during nursery until grafting stage, as well as for successfully grafted seedlings ready to distribution includes watering, weeding, fertilizing and appropriate controlling of pest and disease. Pest and disease control need to be emphasized on

CVPD vector, that is Diaphorina citri and Aphids, besides using sterilized agricultural equipment. Certified citrus seedling producers in both locations had been trying to optimally maintain the seedlings during the production process. Therefore, the adoption level of this particular recommendation was in the category of very high (Table 2).

According to the study results, 80% of the producer sample managed to generate ≥90% of successful grafting and the remaining managed to generate <90% of successful grafting. However, when it came to successfully grafted planting material, only 40% of the producer managed to generate ≥90% of successfully grafted seedling, and the other 60% managed to generate <90% of successfully grafted seedling.

In general comparison, the adoption level of most technology components in Purworejo was relatively higher than Sambas. This might be affected by the level of understanding and awareness of the producers towards the importance of performing recommended production technology.

Factors that might influence the adoption of production technology of citrus seedling was the understanding of citrus seedling producers on the prospect of technology recommendation, intensive guidance on the implementation of recommended technology, and consistency of the regulation implementation [1]. In addition, demographic factors of citrus seedling producers such as education and age might also influence the understanding and awareness of the producers, as producers in Purworejo had the younger profile and higher level of education than those of Sambas.

Meanwhile, the effectiveness of the technology components was also influential in the adoption of a technology [7]. Furthermore, there were two conditions required for technology adoption, i.e., technology dissemination as necessary condition, and institutional support as sufficient condition [7]. Therefore, upgrading the knowledge and understanding of citrus seedling producers about the recommended technology, and encouraging consistent implementation of the technology is important and necessary to be done.

Conclusions

Component of recommended technology for disease-free citrus seedling production that had a very low adoption level in both study locations was the use of polybags during the whole production process. While in comparison, the adoption level of other technology components in Purworejo was relatively higher than Sambas. In Sambas, several technology components of sowing rootstock seed correctly, nucellar selection, transplanting and grafting were still low adopted. In Purworejo, all technology components other than the use of polybags had been highly adopted. Further efforts in improving the capacity of citrus seedling producers, such as training and technical guidance in an effective way are necessary to be done. Thereby, they would adopt the recommended technology better, and eventually, the adoption level of the technology could be increased.

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Farmer Preference on Shallot Cultivation Off Season in Gunungkidul Dry Land at Yogyakarta Special Region

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Abstract

Shallot (Allium cepa var ascalonicum L) is one of the high value economic commodities and it has high risk in off season cultivation. This study aims to determine the preferences of farmers on 4 shallot varieties (3 superior varieties: Bima Brebes, Bauji, Batu ijo) and 1 local variety from Gunungkidul (Saptosari) which were planted off season using silver plastic mulch black, bioslury fertilizer from biogas waste and silicate granule fertilizer as a component assembly on off season shallot cultivation technology. The research was conducted at Karangrejek, Wonosari, Gunungkidul from September to December 2017. Preference assessment based on plant height parameter, number of tillers and plant age. The preference test was done at the end of the activity (before harvest). The data obtained were analysed using Friedman Test with a rating scale of 1 to 5; namely: 1 = very dislike, 2 = do not like, 3 = rather like, 4 = like, 5 = very like. Assessment results were determined from the average score on the 5 highest/best ratings of respondents. Based on crop performance, it appears that the treatment of farmers with Bima Brebes (DV1) varieties was chosen first based on plant age. However, in terms of number of tillers and plant height, C treatment (organic fertilizer + silicate + mulch) on Bima Brebes (CV1) varieties and local varieties become farmers choice (CV4). This suggests that the new technology introduced becomes a farmer's choice based on crop flavour.

Keywords: preferences, shallot, off season and dry land

Introduction

Shallot (Allium cepa var ascalonicum L) was one of the high economic value commodities.

The demand for fresh shallots for household consumption and raw materials for the processing industry continues to increase. On the other hand, the availability/supply of shallots have not yet been able to meet the increasing demand. Therefore, the productivity of shallots needs to be increased. Off-season shallot has become the government's attention in its development efforts which are expected to be able to overcome the provision of shallot production throughout the year.

Planting shallots in the rainy season, which starts from October/December to March/April in normal climatic conditions is usually called off-season plants according to Suwandi (2013).

On the other hand, the contribution of paddy fields in the supply of shallot needs has diminished, so that the fulfilment needs to be supported by the expansion of planting areas.

Yogyakarta Province in its efforts to use relatively narrow agricultural land and land conversion. The project of making marginal land (dry land) as agricultural land is expected to contribute to income for the welfare of farmers.

In Yogyakarta Special Region (DIY), shallot has been proven to provide additional income for farmers (Agriculture Department of Yogyakarta, 2014). Off-season shallot cultivation on dry land was a technological breakthrough to improve the welfare of farmers, because shallot farming in rice fields during the rainy season (off season) was considered inefficient and unprofitable.

The planting of off-season shallots on dry land has not been able to provide optimal yield/production. The low productivity is caused by the high risk, among others: (1) the characteristics of the soil are infertile, formed from weathering of limestone, and problem in the availability of water (2) the low level of application of recommended technology by farmers, (3) an increase in the type and intensity of pest and disease attacks.

Dry land has low soil fertility characteristics. One effort that can be done to overcome this is by giving Bio-slurry organic fertilizer. Bio-slurry is the final product for processing waste made from cow dung which is very useful as a source of nutrients for plants. Bio-slurry fertilizer also contains "biotic" microbes that can improve the physical structure of the soil, improve soil fertility and health of agricultural land so that it is expected to have an impact on improving the quality and quantity of crops (Blue Team, 2012).

In addition, the problem in the cultivation of shallots off season on dry land is the attack of pests (Alternaria sp. and Antraknose sp.,). Weak plant resistance to pest attack caused by low silicate (Si) content of plants as a result of low input of silicate elements (Makarim *et al*., 2007).

So, the use of silicate granule fertilizer is one of the efforts to control pest attacks. Silicate granules are formulated fertilizers containing SiO2 20-22% and P2O5 10-12%. Although not including plant nutrients, Si can increase production because Si is able to improve the physical properties of plants and affect the solubility of P in the soil (Roesmarkam and Yuwono, 2002).

The use of black silver plastic mulch is an important technological component in the cultivation of shallots in the off-season. The results showed that the use of various mulch had an effect on the growth component, namely plant height, leaf number, leaf area, fresh weight of plants and fresh tuber weight (Larmord, 1997).

This study aims to determine the preferences of farmers on the performance of 4 shallot varieties, namely 3 superior varieties (Bima Brebes, Bauji, Batu ijo) and 1 Gunungkidul local variety (Saptosari) which are grown out of season (off season) by using silver black plastic mulch, bioslury fertilizer and granule silicate fertilizer as the assembly of shallots cultivation.

Material and Method

The research was conducted in the "Tirtosari" Farmer Group, Karangsari, Karangrejek Village, Wonosari, Gunungkidul in September to December 2017. The determination of the location was purposive. Four red shallot varieties are 3 superior varieties (Bima Brebes, Bauji, Batu Ijo) and one Gunungkidul local variety (Saptosari) which are grown out of season (off season) using silver black plastic mulch, bioslury fertilizer and silicate granule fertilizer as assemblies of off-season shallots cultivation technology components (presented in Table 1).

Table 1. Technology Components of off-season shallot cultivation on dry land

No.	Shallots varieties (first factor)	Fertilizer dosage (second factor)
1.	Trisula (V1)	Solid Bio Slurry + Inorganic Fertilizer + plastic mulch (A)
2.	Pancasona (V2)	Solid bioslury + anorganic fertilizer + silicate granule + plastic mulch (B)
3.	Bima (V3)	Organic fertilizer + anorganic fertilizer + silicate granule + plastic mulch (C)
4.	Local Gunungkidul (Saptosari) (V4)	Farmers' habits (D)

The design was used a Completely Randomized Group arranged in factorial with 3 replications.

The first factor was 4 shallot varieties and the second factor are fertilizer dosage. The plot size of each treatment combination was 1 x 10 m2.

The research method was used demonstration plots with farmers active participation methods.

Preference test through a survey of 35 respondents on three parameters: plant height, number of tillers and plant age. Preference test was done at the end of the activity (before harvest). The data obtained were analysed using Friedman Test with a rating scale of 1 to 5.

Formula of Friedman test (Sunyoto dan Setiawan, 2013) =

$$x_r^2 = \frac{12}{nk(k-1)} \sum_{j=1}^{k} (Rj)^2 - 3n(k+1)$$

n = the amount of data for each sample
k = number of samples studied
Rj = number of levels per sample
j = sample sequence index to 1, 2, 3, ... j

The results of the assessment were determined from the average value of the 5 highest/best respondents' choices. Preference test uses a preference test (hedonic test) with a level (scale) of preference 1 to 5 (1 = very dislike, 2 = dislike, and 3 = rather like, 4 = like and 5 = really like (Roesmarkam and Yuwono, 2002). Data from the assessment of the level of farmers' preference were analysed statistically further using Friedman Test (Sunyoto and Setiawan, 2013). Friedman Test analysis using SPSS ver 16.

Result and Discussion

Preference tests were carried out to determine the preference/level of respondents' preference for the performance of plants in some shallots grown off-season on dry land by using assemblies of technological components. Farmers' preference for plant performance was carried out by direct observation in the field, while the assessment method was giving a score on a scale of 1 to 5.

Furthermore, this scoring data was analysed using Friedman Test, the results of the analysis are presented in Table 2.

Table 2. Results of the analysis of plant preference test

	Plant height	Rank	Number of tillers	Rank	Age	Rank
AV1	6.67	14	6.53	15	5.89	16
AV2	7.33	12	9.49	6	8	10
AV3	5.46	16	4.79	16	6.24	15
AV4	7.59	9	8.67	8	8.99	8
BV1	9.06	7	10.06	4	7.51	12
BV2	6.70	13	6.94	13	7.96	11
BV3	11.33	2	7.31	11	10.3	4
BV4	7.59	10	9.49	7	9.07	6
CV1	9.91	5	10.11	3	10.13	2
CV2	10.61	3	9.94	5	9.81	5
CV3	8.5	8	7.03	12	8.49	9
CV4	12.23	1	11.79	1	10.09	3
DV1	10.33	4	11.33	2	10.27	1
DV2	9.07	6	8.06	9	9.03	7
DV3	7.43	11	7.6	10	6.99	14
DV4	6.2	15	6.87	14	7.24	13
N	35		35		35	
Chi Square	107.25		101.876		105.682	
df	15		15		15	
Asymp sig	.000		.000		.000	

Information: Solid Bioslury + Inorganic fertilizer (A)
SolidBioslury + inorganic fertilizer + silicate granule (B)
Organic fertilizer + inorganic fertilizer +Silikat granule (C)
Famer's habit (D)
Trisula (V1)
Pancasona (V2)
Bima (V3)
Local Gunungkidul (Saptosari) (V4)

Table 2 showed that respondents' preferences on plant height statistically have a significant difference, this is seen from the value of Asymp.Sig. Less than 0.05. The preference/level of respondents' preference for plant height parameters sequentially was CV4 (12.23), BV3 (11.33), CV2 (10.61), DV1 (10.33) and CV1 (9.91). The preference/level of respondents' preference for the parameters of the number of tillers are selected CV4 (11.79), DV1 (11.33), CV1 (10.11), BV3 (10.06) and CV2 (9.94). The preference/level of respondents' preference for plant age parameters in sequence is DV1 (10.27), CV1 (10.13), BV3 (10.3), CV4 (10.09) and CV2 (9.81).

Based on the results of consumer preference test for plant performance, five varieties and treatments selected for plant height parameters, number of tillers, plant age are presented in Table 3.

Table 3. The five best treatments based on consumer/farmer preference for the performance of plants (plant height, number of tillers, and plant age)

Rank	Treatments		
	Plant height	Number of tillers	Plant age
1.	CV4	CV1	DV1
2.	BV3	DV1	CV1
3.	CV2	CV1	CV4
4.	DV1	BV1	BV4
5.	CV1	CV2	CV2

Table 3 showed that based on the results of the respondents' choices in each parameter, the selected varieties in order are V1 (Bima Brebes), V4 (Lokal Saptosari), V2 (Bauji) and V3 (Batu Ijo). Respondents' choice of shallot varieties is certainly based on plant performance which was the genetic expression of each environment-influenced variety. The character of shallots bulbs was influenced by genetic factors and little by environmental factors (Budianto *et al.*, 2009). The number of tillers has a relationship with the size of onion bulbs. Shallots that have a large bulb size will have a smaller number of tillers (Basuki, 2005 dalam Kusmana *et al*., 2009). Therefore, the selection of the right variety was one of the important technological components in each farm.

That the main key to cultivating shallots outside the season was the selection of varieties (Purba and Astuti, 2013; Purba, 2014).

According to respondents, the choice of preference for varieties V1 (Bima Brebes) and V4 (Local Saptosari). The choice of preference in V1 (Bima Brebes) was due to the good performance of the plants (DV1, CV1, BV1). The choice of respondents' preference in V1 (Bima Brebes) because Bima Brebes variety was chosen by participant farmers as the most preferred variety, due to simultaneous plant growth, fertility of leaf growth, plant height, leaf shape and colour, and leaf thickness and width (Basuki, 2009). According to farmers' perceptions, the best quality shallot variety is Bima Brebes. All quality attributes possessed by Bima Brebes according to farmers' judgment were above average. The tuber size, spiciness, tuber colour, number of tillers, and yields of Bima Brebes were 4%, 28%, 27%, 11% and 16% respectively above mean (Basuki *et al*., 2014).

The choice of the second preference for V4 variety (Local Saptosari) was due to the large number of tillers (CV4=11.7). Saptosari variety was local variety that have been adaptive in Gunungkidul so the production was high. Its good performance will cause high production.

Production is the most important attribute according to farmers, because if production is high, farmers' income will also be high (Rahayu, 2012).

Whereas the technology innovation of off-season shallot cultivation on dry land based on bioslury fertilizer and silicate granules that farmers like in sequence is treatment C (organic + an organic + silicate + plastic mulch), treatment B (Bioslury + an organic + silicate + plastic mulch) and treatment D (organic + organic without plastic mulch).

The large number of tillers from CV4 favoured by these respondents is in accordance with the results of the Bioslury Fertilizer Usage Study of Biogas Waste and Silicate on Off-season Shallot Cultivation in Dry Land. In the report of the study, Wiranti (2017) stated that the genetic potential of each shallot variety planted was influenced by the environment as a result of Bioslury and silicate fertilizers. The CV4 treatment gave the highest productivity (27.08 tons/ha of wet bulbs).

Treatment of C is the treatment of organic fertilizer and silicate with local varieties of Saptosari (V4). The provision of organic fertilizer and silicate can improve plant growth and shallot production. Hardjowigeno (1989) states that the main requirement to overcome nutrient deficiencies in soil is the improvement of soil structure through organic fertilization. Organic fertilizers that have undergone a decomposition process can suppress the growth of disease in the soil and act as a nutrient provider for plant growth in the form of organic compounds.

Giving plastic mulch was the choice of farmers because of the many benefits including suppressing weeds, resisting high rainfall directly into plants (because it was planted off-season/in the rainy season). Sunlight that was passed through the surface of the mulch is trapped on the surface of the soil it covers and forms a "greenhouse effect" on a small scale. This trapped heat will increase the surface temperature of the soil, modify the balance of ground water, reduce soil weeds, and increase microorganism activity (Waggoner, 1960; Tanner, 1974; 15 Mahrer *et al*., 1979) in Fahrurrozi (2009).

Conclusion

1. Shallot varieties favoured by farmers in off season shallots cultivation on dry land in sequence are V1 (Bima Brebes), V4 (Lokal Saptosari), V2 (Bauji) and V3 (Batu Ijo).
2. Technological innovation of off-season shallots cultivation on dry land based on bioslury fertilizers and silicate granules that farmers like in sequence are C (organic + an organic + silicate + plastic mulch), B (Bioslury + an organic + silicate + plastic mulch) and D (organic + an organic without plastic mulch).
3. Implications: It is expected that from the results of this study farmers can adopt off-season shallots cultivation technology on dry land according to farmers' preferences.

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Analysis of Competitive Assessment of Consumer Requirements and Technical Requirements on The Quality Development of *Dendrobium* Orchid Potted Flowers

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Abstract

Dendrobium is a popular orchid among other types of orchids. This ornamental plant is also in demand by the market. Unfortunately, the increase in production is not able to meet the demand.

To reduce the risk of producing products that are not in accordance with consumer preference, a development effort is needed to improve the quality of new varieties of Dendrobium pots flower based on consumer preference. This study aims to (1) analyse the attributes of the Dendrobium pot flower from the consumer side (2) analyse the attributes of the Dendrobium pot flower from the producer side. The data used in this study were primary and secondary data. This study used the analysis method of Quality Function Deployment. The results showed that the Dendrobium pot flower attributes desired by consumers were bright flower colours, healthy plants, sturdy and compact, erect stems with large numbers of flowers, high frequencies in flowering and sprouting, easily cultivated. While the Dendrobium pot flower attributes desired by producers were good plant conditions, quality flowers, ease of obtaining seeds, ease of production, pest and disease resistance, purchase price of seeds, selling price of interest prices. The implication of this research is as a guide to improve the quality development of the varieties. A solution to overcome the availability of Dendrobium potted flowers seeds are tissue culture technology supported by advanced technology (SOP and GAP based cultivation technology) and government support, especially regarding reliable information systems and cooperative relations between research institutions and stakeholders.

Keywords: attribute consumer side, producer side, quality development, Dendrobium orchid potted flowers

Introduction

Floriculture ornamental plants are one of the horticulture sub-sector which is highly potential to be developed as one of national income sources in the future. Countries that have also begun to become exporters are Thailand, Malaysia, Australia, Israel, New Zealand, and South Africa. Data showed that an increase in the export volume of orchid plants was 22.75 percent over the period 2015-2016 [1].

Dendrobium is one of the largest orchid genera from the family of Orchidaceae. The orchid genera are an important genetic resource, which is vastly available in the eastern Indonesia. Seed is one of the keys to success in the agribusiness. The usage of superior seed varieties determines the success of orchid productions. Recently, the quality of seeds available on the market is rather low. Imported seeds are considered necessary since domestic seeds is deemed inadequate. Efforts are necessary to increase the quality, availability, and usage in floricultural agribusiness.

The Dendrobium industry in Indonesia has not developed because of the quality, quantity and continuity of seed production, which is considered low. Indonesia has a diversity of genetic resources in the form of various orchid varieties. The diversity of orchid varieties is a potential in cross-breeding to produce characteristics of varieties that are suitable for consumer needs [2].

Dendrobium is extremely popular among orchids.

In general, quality and service play an important role in differentiating products. Marketing aims to fulfil customer desires and needs, which in turn, improve product quality based on various feedback received from customers. Marketing is a complex problem, with many challenges to predict and understand what customers want. Producers value all necessary information regarding what is demanded by consumers in order to research new products, features, distribution price, messages, and any other marketing elements. [3]

Argues that farmers consider the superiority of the overall characteristics of varieties to be a major factor in their decision making. These characteristics are the shape, size, root colour, and high adaptability so that the variety can be planted during the dry and rainy season. [4]

Explained that the application of quality standards on frozen edamame products for the international market was carried out by determining the critical points, namely pesticide residues, blanching, cold storage and metal detectors. Another statement explains that the conditions expected by consumers about priority attributes can be uniform and diverse. [5] describes seven attributes that are priorities in choosing vegetables, namely freshness, cleanliness, colour, shape that is in accordance with standards, uniform size, durability and guarantee of food safety. This study aims to (1) analyse the attributes of the Dendrobium potted flowers from a consumer perspective, and (2) analyse the attributes of the Dendrobium potted flowers from the perspective of the producer.

Materials and Methods

This research was conducted in Taman Anggrek Ragunan and Taman Anggrek Indonesia Permai since both locations are the commercial centers of orchid potted flowers in Jakarta. The number of respondents were 35 farmers who users of Dendrobium orchid potted flowers new varieties and competing superior varieties, while the experts were breeders from Indonesian Ornamental Crops Research Institute (IOCRI), the orchids association of Indonesia in Jakarta.

Research was conducted in June-August 2016.

This research used primary and secondary data. The primary data was taken with a method of surveying in the form of interview based on the questionnaire. Secondary data as supporting data obtained from various agencies among others central bureau of statistics, the ministry of agriculture, IOCRI, the journal, internet as well as literatures related to the research topic. Method of analysis used quality function deployment (QFD). QFD is a planning used to conformity the hopes of consumers on a product. QFD is a way to improve the quality of goods or services by understanding the needs of consumers, then connecting with technical provisions to produce goods or services at each stage.

The QFD method is more advanced than the analysis of consumer preferences, because in the QFD structure customer desires information is accommodated in the ability of production planning techniques. The QFD method begins with compiling the House of Quality (HOQ) matrix. The HOQ matrix is a product planning matrix which translates consumer requirements into a number of technical targets so that products produced will be able to meet consumer desires [6], [7] explained that HOQ was set to meet consumer needs. This stage is the most crucial point in determining quality.

Steps in the preparation of the HOQ matrix of Dendrobium Orchid Potted Flowers can be seen in Table 1

Table 1. House of Quality Matrix Preparation of *Dendrobium* Orchid Potted Flowers

Matrix	***Consist of:***	***How to Obtain:***
A	Consumer's Requirements *(What),* the HOQ input	Market Survey
B	a. Weighting the importance of consumer needs b. Consumer satisfaction level towards service product c. Consumer satisfaction level towards similar products or services from competing brands	Comparing products that will be developed with competing units, including consumer's interest, target value, scale increment factor, sales point, absolute weights, priorities of consumer's requirements
C	Technical requirements toward new varieties that will be developed (how)	Interview with producer/development team.
D	Relation power between matrix A and C	Explain the relationship between what and how, described as *strong*, *adequate*, and *weak*
E	Correlation between one technical requirement and others (matrix C)	Explain the relationship between technical requirements, described as *positive*, *negative*, and *0*
F	a. Technical requirements priority rank b. Information to compare technical performance of product service produced toward competing units c. Technical requirements performance target of newly developed product or service	An analysis of technical requirements, including: competitive assessment of product that will be developed against competitors, difficulty degree, technical target value, absolute weight and its priority, relative weight and its priority

Results and Discussion

Technological advances require producers to be able to produce products that are better, easier, superior, and in accordance with consumer needs. Technology has a major role in developing new products. However, quality is what will determine whether the new product is preferred by consumers or not.

Referring to the concept of customer satisfaction, producers need to conduct research and development based on customer satisfaction. Therefore, the process of designing new varieties is more than what benefits will be fulfilled, but also the design, name, trademark, warranty, product image, and customer service. In the end, there are no varieties that can be described as perfect varieties.

Consumer Requirements for New Varieties of Dendrobium Orchid Potted Flowers

The QFD method is done by adjusting between consumer desires and the technical requirements of the producer. [8] explains that the customer's assessment of each requirement is to keep the product unchanged, improve the product, and make the product better than its competitors. [9] explained that the QFD approach can guarantee that the quality of the prolate entering the production stage can truly satisfy customer needs. Quality improvement is carried out based on customer expectations in the order of priority.

Dendrobium Orchid Potted Flowers are vastly diverse, whether by its colour or shape. Changes in Dendrobium orchid potted flowers are more dynamic and has more demands, compared to cropped ones. Dendrobiums potted are also used as garden or indoor décors.

The research results showed that consumers of Dendrobium pot wanted varieties that were easy to obtain and cultivate, bright, stung, upright, proper length, high number of bud, bloom, germinate frequently, thick sepal and petal, and compact-sized as listed in Table 2.

Table 2. New Verities of *Dendrobium* Orchid Potted Requirements According to Consumers

Primary Requirements	***Secondary Requirements***	***Competing Varieties****
Availability	Seeds availability (very easy)	Seeds availability (very common)
Farming difficulty	Farming difficulty (easy)	Cultivation difficulty (easy)
Flower colour	Colour of flower (bright)	Colour of flower (vibrant, high amount of motives)
Sprig strength of peduncle	Strong, upright, proper length	Strong, upright, proper length
Number of bud	High number of bud	High number of bud
Flower productivity	Bloom and germinate often	Bloom and germinate often
Flower resiliency	Thick sepal and petal	Thick sepal and petal
Appearance	Compact-sized (overall)	Compact-sized (overall)

Source: The Primary Data are the results of interview with farmers of Dendrobium orchid potted flowers

Preparing the Producer Technical Requirement

Technical requirements are prepared based on information obtained from consumer demand.

The purpose of this compilation is to translate consumer requirements into technical steps to obtain the suitability of developed products with consumer demands. Technical requirements are the result of opinions from experts [10].

The technical requirements are based on interviews with dendrobium pot farmers, which consist of primary and secondary technical requirements. Primary requirements consisting of: physical traits of dendrobium (agronomical characteristics), cross-breeds, cross selections, seed multiplication technique, advanced test technique, resiliency against pesticides, and government support. Secondary requirement included in physical traits (dendrobium's agronomical characteristics) is the number of pseudobulb or productive bulbs per plant. Secondary requirement included in cross-breeds is the colour of flower, length of sprig, number of bud per sprig, and sprig traits. Secondary requirement included in cross selections is assessment of flower traits and quality. Secondary requirement included in seed multiplication technique is in vitro. Secondary requirement included in advanced technique are Cultivation method based on Standard Operational Procedures (SOP) and Good Agricultural Practices (GAP), post-cultivation method based on GHP (Good Handling Practices). Secondary requirement included in government support are powerful information system and partnership between research institutions and businessmen.

These technical requirements and its measurements are listed in Table 3.

Table 3. Technical requirements of new varieties of *Dendrobium* orchid potted flowers

Technical requirements		***Unit of measurement***
Primary	***Secondary***	
Physically Characters of plant	Characters of plant agronomy	Bulb
	flower colour	-
	flower size	-
	panicle length	Cm
	inflorescence stalk length	Cm
	bud number per inflorescence stalk	petals
	inflorescence stalk characters	-
Hybrid selection	assessment of the character and quality of the flowers	-
Seed technology	in vitro	-
Technology	cultivation technology based of SOP and GAP	-
	Post-harvest technology based of GHP	-
Cultivation	Fertilizer responsive	-
	Pest and disease resistance technology	-
Government support	Reliable information system	-
	The relationship of cooperation between research institution and stake holders	-

*Source: *The primary data are interview result with the breeders and the expert of Dendrobium potted flowers from IOCRI*

Competitive Assessment

Competitive assessment is split into two parts: consumer competitive assessment and technical competitive assessment. Consumer Competitive Assessment is a method to determine whether the consumer requirements are met, and identify which consumer requirement should be given more priority in the next development and improvement of varieties.

Technical Competitive Assessment is done by comparing the technical requirements to produce a new variety against its competitors. Both assessments require the new potted dendrobium to be compared with competing top breed dendrobium, which is the most dominant variety available on the market today. These comparisons are made since customers prefer varieties that are easily available, easy to cultivate, large size, vibrant colours and motives, strong sprigs, and bloom often.

Table 4. Consumer Competitive Assessment

Consumer Requirements	***Answer***	
	D. VB	***D. Competitor***
Seed availability (very easy)	1	3
Cultivation difficulty (very easy)	1	3
Flower color (bright)	3	4
Sprig strength of peduncle (strong, upright, proper length)	2	4
Number of bud (high)	4	4
Flower productivity (often bloom and germinate)	3	4
Flower thickness (thick sepal dan petal)	2	4
Appearance (compact)	3	4

Description: 1 = very not good, 2 = not good, 3 = good enough, 4= good, 5 = very good

Technical Competitive Assessment

Competitive assessment is split into two parts: consumer competitive assessment and technical competitive as This assessment is done by comparing the product with its closest competitor for every technical requirement, using five-point Likert scale. The result can be seen in Table 5.

Table 5. Technical Competitive Assessment

Technical Requirements		***Answer***	
Primary	***Secondary***	***D. VB***	***D. Competitor***
Physically characters of plant	Agronomical characteristics	3	3
Plant selection	Assessment of the character and quality of the flowers	3	4
Seed technology	In vitro method	3	3
Advanced technique	cultivation technology based of SOP and GAP	2	2
	Post-harvest technology based of GHP	3	3
Cultivation	Fertilizer responsive	3	3
	Pest and disease resistance technology	2	2
Government support	Reliable information system	2	2
	The relationship of cooperation between research institution and stake holders	2	2

Description: 1 = very not good, 2 = not good, 3 = good enough, 4= good, 5 = very good

The survey results indicate that the technical requirements that are good or bad compared to competing products are as follows.

1. In vitro methods, responsive fertilizers, GHP-based post-harvest technology, and agronomic characteristics are considered equal or better.
2. Advanced techniques towards resistance to pesticides, reliable information systems, and cooperative relations between research institutions and stakeholders are considered to be equal or worse.

Conclusions

Based on the results of the study, it can be concluded that:

1. In vitro method between new and comparative varieties is the technical requirement considered as good by the breeders of Dendrobium orchid potted flowers.
2. The seed users of Dendrobium orchid potted flowers want availability and ease of cultivation in development of Dendrobium orchid potted flowers.
3. Consumer competitive assessment towards Dendrobium orchid potted flowers research is advanced technique related to breeding technique based on SOP and GAP, government support, especially regarding powerful information system and partnership between research institutions and stakeholders.

Recommendation

A solution to overcome the availability of Dendrobium potted flowers seeds:

1. Tissue culture technology supported by advanced technology (SOP and GAP based cultivation technology)
2. Government support, especially regarding reliable information systems and cooperative relations between research institutions and stakeholders.

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Factors Affecting the Adoption of Certified Citrus Seed

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Abstract

The main problems in citrus cultivation in Indonesia is Citrus Vein Phloem Degeneration (CVPD) diseases which have attacked in most of citrus plants caused by the use of uncertified seed. The objective of this study was to analyse factors that influence the use of certified citrus seeds. This research was conducted in September-October 2017 in Malang, Jember and Banyuwangi as the biggest citrus producer. The sampling method was multistage random sampling with 500 respondents. The analytical method used was binary logistic regression model.

The research result showed that the use of certified citrus seeds was significantly influenced by farming experience, income, and participation in farmer's group. Hence, increasing the use of certified seed can be done by increasing the farmers' participation in farmers group. Socialization of technology needs to be focused on low-income farmers. In addition, intensive dissemination can be given to young farmers, in which can become champion farmers.

Keywords: Adoption, certified, citrus, seed

Introduction

Citrus is the important commodities in Indonesia, in which become one of the five commodities that make the biggest contribution to national fruit production. Citrus ranked fourth with a production contribution around 9.01 percent of national fruit production [1]. During the period 1980-2015 there were fluctuations in citrus production with an increasing trend of 3.94 percent per year. This is in line with the fluctuations in the area of citrus harvests with an increasing trend by 9.94 percent per year. This is resulted from intensification, but its constraints to the limited availability and suitability of land, so that an increase in area cannot be continuously carried out.

The decline in harvested area has occurred over the past ten years, about 12.70 percent per year.

This was followed by a decrease in production by 13.11 percent per year. Therefore, the intensification efforts are needed. On the other hand, there was an increase in citrus consumption during the period of 2006-2015 which was 2.58 percent per year. This causes a trade balance deficit [2]. To overcome this, efforts to increase citrus production are urgently needed.

However, it was facing serious problems, especially because of the attack of CVPD. This disease has become an endemic disease in various area [3]. All citrus plants showing the nutrients deficiency were infected by the pathogenic bacteria, the fruit were physically small, lack of vitamin C and antioxidants [4]. But unfortunately, farmers' understanding of the disease is still lacking. Farmers also have not adopted technology to overcome this disease.

The cause of high CVPD attacks is the use of non-certified citrus seeds. The use of healthy seeds is main components of CVPD control [5]. Certified budwood programs might be the best way to establish disease-free citrus orchards [6]. So far, research has focused on technology to deal with CVPD attacks, assembly of resistant diseases, and the importance of using certified citrus seeds. However, there have not been many studies that examine how the technology can be adopted by the farmers. Therefore, this research become important to analyse factors that influence the use of certified citrus seeds. By knowing this information, the important variables in dissemination process will be obtained and formulated into policy recommendation.

Methodology

This research was conducted in September-October 2017 in East Java. Secondary data was obtained from the Data and Information Center of the Ministry of Agriculture, Directorate General of Horticulture, and related publications. Primary data was obtained through surveys of 500 citrus farmer households with multistage random sampling. The first stage is the Regency election. The selected districts are Malang, Jember and Banyuwangi because the location is the 3 largest citrus production centers in Indonesia. The second stage is the selection of sub-districts, which are production proportion are more than 5 percent in each of the selected districts. The third stage is to randomly select 14 villages and must be distributed to all selected sub-districts. The fourth stage is to randomly select 12 households in each village based on the population data. The number of respondents from Jember and Malang, respectively 166 respondents, while Banyuwangi is 168 respondents.

Factors influencing the adoption of certified citrus seeds were analysed by logistic regression models. It analyses the relationship between multiple independent variables and a categorical dependent variable, and estimates the probability of occurrence of an event by fitting data to a logistic curve [7]. This model is recommended to analyse factors that influence technology adoption [8]. This model is also able to overcome problems that arise in linear probability models (LPM) when the dependent variable is a dichotomy (0 and 1) [9]. Many studies using this model to measure adoption level include [10] who used a logit model to measure socio-economic factors that influence adoption of hybrid corn. In his research, the factors that were included as independent variables were age, income, education, visits of extension workers.

Adopting the theories and research results of [11], [12], [13], [14] and [15], factors that influence the adoption process are represented by individual differences (farming experience, main work), consumer resources (education, income, involvement of family members in citrus farming , number of productive trees), demographics (age, sex), information processing (internet access), and learning (participation in farmer groups, access to government). Logit regression in this study referring to [16] as follows:

P=opportunities for farmers to adopt technology (Pi = 1 if farmers adopt, Pi = 0 if farmers do not adopt)

1 – p1=opportunities for farmers not to adopt technology

X1=Age (year)

X2=education (year)

X3=The involvement of family members in citrus farming (people)

X4=Productive trees ownership (tree)

X5=Farmer experience in citrus farming (year)

X6=Income from citrus farming (Rp)

D1=Gender (D=1, Men; D=0, Women)

D2=Dummy Main Activity (D=1, citrus farmer; D=0; others)

D3=Dummy internet access (D=1, have access; D=0; not have access)

D4=Dummy member in farmer group (D = 1, member; D = 0, not member in farmer group)
D5=Dummy extension agent access (D = 1, have access; D = 0, not have access)
A=Constanta or intersep; βi = i-regression coefficient (i = 1, 2, 3, ... 11)
E=Error term

Results

Farmers Characteristic

The research results showed that majority of citrus farmers in the study regions were in productive age (36-65 years). Although the education level is not considerably high (elementary school educated), but the experience of farming has been long enough (10-20 years). There is a small percentage are still working even though they are at an unproductive age, while the young farmers are the smallest due to low interest of young generation for farming. Therefore, efforts are needed to attract the interest of the younger generation with higher education levels for farming.

The research result of [17] also suggest the need for regeneration in cocoa farming, as many older farmers will retire in the next 5-10 years. In addition, the productivity of farmers appears to increase slightly and then decrease with age, in which enhance technology use occurring at mid-life, although for many stages there is also a slight increase and then decrease in efficiency by age [18]. The farmers characteristics are presented in Table 1.

Table 1. Characteristic of Citrus Farmer in di Malang, Jember dan Banyuwangi

Farmer Characteristic	Banyuwangi (%)	Jember (%)	Malang (%)
Age (year)			
≤35	2,97	2,41	7,23
36-65	80,36	87,35	74,09
>65	16,67	10,24	12,05
Education			
Not educated	22,02	22	26
Basic school	33,33	27	47
Junior high school	17,86	17	9
High school	19,64	28	14
College	7,14	7	4
The involvement of family member			
1 person	24	27	20
2 persons	57	57	52
3 persons	18	13	20
4 persons	1	2	7
5 persons	1	1	0
Experience in citrus farming			
0-10 year	39	21	61
11-20 year	42	25	17
21-30 year	17	45	17
>30 year	2	9	5
Income			
<2 million	21,71	25,66	36,89
2-10 million	26,32	24,34	13,59
10-20 million	23,03	19,74	15,53
20-50 million	17,76	22,37	16,50
50-100 million	6,58	5,26	10,68
>100 million	4,60	2,63	6,79

Citrus farming is also dominated by farmers with low education levels, most of whom do not graduate from elementary school and do not go to school (Banyuwangi and Malang), a well as graduated from elementary and high school (Malang). Low level of formal education resulted in low level of adoption technology level. Education increases the ability to assess, interpret and process information about technology, enhance farmers' managerial skills, including efficient use of agricultural inputs [19]. Education has an important role to play in increasing agricultural production, which is productivity may be enhanced either through the adoption of more productive inputs and techniques or through improvements in productive efficiency for a given technology [20].

The average number of family members involved in citrus farming is 2 people. In terms of farming experience, most farmers in have experience of 11-20 years (Banyuwangi), 21-30 years (Jember), 0-10 years (Malang). This implies that majority of citrus farmers having a long period. Management skills of the farmer improved with experience [19].

The average income earned during the year is 35 million rupiah. The income gap from citrus farming in Banyuwangi and Jember is low, with most farmers receiving income of 2 million to 50 million rupiah in almost the same proportion. Meanwhile, most farmers in Malang have incomes of less than 2 million rupiah.

Factors Affecting Adoption Certified Citrus Seed

The results of multicollinearity testing showed that there is no strong correlation between variables. This is indicated by the absence of a test result value of more than 0.8. The results of the multicollinearity test are presented in Table 2

Table 2. Results of Multicollinearity Test

	Intern~s	Gender	Age	Educat~n	MainAct	HH_siz~s	Produc~e	Experi~e	Farm_G~p	Access~v	Citrus~c
Internet_a~s	1.0000										
Gender	-0.0146	1.0000									
Age	-0.2238	0.0338	1.0000								
Education	0.2960	-0.0862	-0.4025	1.0000							
MainAct	0.0089	-0.0689	-0.1979	0.1278	1.0000						
HH_size_ci~s	0.1011	-0.0987	0.2140	-0.1967	-0.0421	1.0000					
Productive~e	0.1282	0.0000	0.0800	0.1284	-0.1500	0.0057	1.0000				
Experience	0.0825	-0.0640	0.2634	0.0094	-0.0598	0.0526	0.1118	1.0000			
Farm_Group	0.1283	-0.0125	0.0683	0.0066	-0.0215	0.1631	0.0303	0.1216	1.0000		
Access_Gov	0.1280	-0.0787	-0.0571	0.2091	0.0532	-0.0122	0.0027	-0.0261	0.1416	1.0000	
Citrus_Inc	0.1242	-0.0087	-0.0164	0.0770	-0.1030	0.0237	0.4822	0.0809	0.1918	0.0333	1.0000

The test results of simultaneous test showed that with a confidence level of 95 percent, the probability of a statistical LR is 0.0000 so that H0 is rejected. This can be seen from the value of LR Chi (2) of 40.85 with Prob>chi2 of 0.0000. In other words, the eleven variables simultaneously affect the adoption of certified seeds.

The results of goodness of fit test through the person or hosmer lemeshow test show the Prob> chi2 value of 0.2131. The value of Prob>chi2 which is greater than 0.05 indicates that the model is considered able to predict the observation value or the model is acceptable because it matches the observational data.

The results of partial test showed that of the eleven variables tested, there were 3 variables that significantly affected the adoption of certified citrus seeds, namely the experience of citrus farming, income and participation in farmer groups. This is indicated by the Prob>chi2 value of the three variables which is <0.05. Table 3 shows the results of partial significance tests on factors that influence adoption of certified citrus seeds.

Table 3. Partial Significance Test of Factors Affecting Adoption of Certified Citrus Seeds

No	Variabel	Prob>chi2
1	Age	0,8467
2	Education	0,4250
3	The involvement of family member in citrus farming	0,2625
4	Productive citrus tree	0,8709
5	Experience in citrus farming	0,0008
6	Income	0,0264
7	Gender	0,1080
8	Main Activity	0,5343
9	Internet Access	0,2462
10	Member in farmer group	0,0000
11	Government access	0,4752

The three variables that have significant influence were then further tested by measuring the coefficients and the odds ratio. The test results showed that the variables that have a positive effect are income and participation in farmer groups. The income variable has an odd ratio of 1 meaning that if the income increases by 1 rupiah, the opportunity to adopt certified citrus seeds increases in the same proportion. This is because the level of income is related to the area of land owned, the ability to access credit, the ability to bear the risks and the ability to access information.

Farmers who have high income levels have a greater chance of accessing information, especially regarding the importance of adopting certified citrus seeds. In line with the results of this study, [21] argue that income is also one of the factors that influence the adoption of superior varieties of white corn. Therefore, the socialization of technology needs to be focused on low-income communities. This finding is in line with [22] and [19] who stated that there is a positive relationship between income and the level of adoption.

The variable member in farmer groups has an odds ratio of 3.34, meaning that farmers who are members of farmer groups have a chance of 3.34 times higher than those who do not member in farmer groups. This is because farmers who are members of farmer groups have a greater chance to connect with extension workers, so the opportunity to adopt technology is greater. Farmers who have frequent contacts with extension agents have a higher probability of participation in the innovation. [23] stated that contact with extension workers was found to be positive in determining adoption. Government extension workers play an important role in such activities, so that increasing the propensity to be aware of improved technologies [24]. This was presumed, as farmers were privileged with maternal and managerial support, followed by cheap and timely availability of knowledge and skills, which apparently helped them apply new technology [10]. In addition, membership of associations enables farmers to interact with other farmers, share their experiences, and assist themselves. Interaction with farmers is an avenue through which innovation diffusion can occur [19].

The variable that has a negative effect is the experience of citrus farming. The experience of citrus farming has an odd ratio of 0.96, meaning that if the farming experience increases by one year, the chance to adopt certified orange seeds decreases to 0.96 times. This is because experienced farmers are accustomed to using cultivation technology that has been carried out for generations, including in terms of seed selection, so they tend to feel they do not need to adopt new technology. Similar finding was reported by [25]. They said that the coefficient of year of experience was negative and that farmers were significantly influenced decision to adopt improved technology in Tanzania. Consumers who have a lot of knowledge and experience tend not to be motivated to seek information because they feel sufficient with their knowledge in making decisions [13]. Therefore, experienced farmers tend to have difficulty adopting recommendations to use certified citrus seeds.

Conclusion

The research result showed that the use of certified citrus seeds was significantly influenced by farming experience, income, and participation in farmer's group. Hence, increasing the use of certified seed can be done by increasing the farmers' participation in farmers group. Socialization of technology needs to be focused on low-income farmers. In addition, intensive dissemination can be given to young farmers, in which can become champion farmers.

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The Performance of Pineapple Supply Chains in Riau Province, Indonesia

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Abstract

This study aimed to identify the actors involved in the pineapple supply chain, identify their roles, analyses the chain's governance structure and formulate alternative strategies to link small-holders to the modern market in Riau Province. The study was conducted during January-December 2016, in Kampar District. Primary data were collected through field observations and interviews, while secondary data were obtained from relevant institutions. Data were analyzed using the principles of supply chain analysis. The results showed that there were several actors involved in the pineapple supply chains in Riau. The governance structure among actors along the chain was mostly in the form of informal contracts. The number of modern retail markets and food processors is increasing in Riau, providing new market opportunities for pineapple farmers.

However, to participate in such chains, farmers still face several challenges, including meeting the higher quality requirements imposed by modern markets, managing the payment delays and costs associated with supply to supermarkets and ensuring supply continuity.

Keywords: pineapple, supply chain, Riau, Indonesia

Introduction

Increasing income, urban population growth, policy change and the liberalization of food industry have transformed the agri-food supply chain in developing countries [1]. Higher disposable income has made a significant change in the willingness to pay more for healthier food.

Further, it has motivated a dramatic shift in diets, away from carbohydrates to healthier food choices, including vegetables and fruit. In addition, these changes have made consumers more selective in their food choices in terms of variety, quality, standards, uniformity, brand and other product attributes [2]. These conditions, together with liberalization of foreign direct investment, have motivated the development of modern markets, including food processors and modern retail markets, in developing country domestic markets.

In order to fulfill consumer demand for higher quality food, a 'supermarket revolution' has occurred in developing countries over the past two decades. This revolution has included continuity and innovation transformation, as well as unique development strategies [3]. As modern markets develop, small-holders face greater choices and daunting decisions about which crops to produce, how to produce and who to sell to [1]. This rapid transformation presents small farmers, including pineapple farmers, with new and potentially profitable opportunities to participate in modern fresh fruit and vegetable chains [2]. Pineapple is Indonesia's top horticultural crop, and is targeted to contribute to national development through the National Agriculture, Fisheries and Forestry Rehabilitation Programme [4].

Previous studies on pineapple supply chains include [5], which mapped and analyzed the fresh pineapple supply chain in Benin, to identify bottlenecks in pineapple quality improvement for different markets. Chain diagnosis showed that there was no concordance between actor groups regarding which quality attribute they valued most. Moreover, pineapple quality was found to be highly heterogeneous. Key bottlenecks were attributed to the lack of training of primary producers in production practices, air-conditioned transport and boxes for export. The lack of good and timely planting materials and skilled labour, electricity fluctuations and ineffective pre-cooling and cold chain facilities were the major risks facing pineapple supply chains in Ghana, and recommended investing in in-vitro planting facilities, staff development and continuous improvements through modernization and re-tooling [6].

However, little research has been conducted on peatland agroecosystem, in which pineapple is grown in Riau, a major pineapple production centre in Indonesia. This province has suitable agro-ecological conditions to grow pineapple. By growing pineapple, farmers might earn higher income than cultivating rice or even palm oil, as the major soil type in Riau is peat soil. The average pineapple production during 1980-2014 in Indonesia was 16.51% per annum [7]. This study is valuable, as it aims to support the participation of small-scale pineapple farmers in modern markets, contributing to the development of the Riau regional economy.

Initially, pineapple farmers sell their pineapple fruit in the traditional markets. As modern market was developed, pineapple farmers have the opportunity to expand selling and marketing of fresh pineapple into modern markets. Therefore, this study aims to examine the actors involved in the pineapple supply chain, identify their roles, analyses the chain's governance structure and formulate alternative strategies to link small-holders to the modern market in Riau Province.

Methodology

Study Area

Kampar District was chosen as the study area, for the following reasons:

- It is the second biggest pineapple producer in Riau Province (after the city of Dumai).
- It has a large number of pineapple growers and household industries that process pineapple into processed products.
- Kampar is accessible, and has the appropriate infrastructure to support pineapple industry development, such as good quality roads and sea ports (Port of Sungai Duku and Port of Pelita Pantai).
- Because of budget limitations: Kampar District was considered suitable as it is close to Pekanbaru, the capital city of Riau.

Types of Data and Sampling Technique

The data collected in this study consist of primary and secondary data. Primary data were collected by field observations and interviews of 30 farmers and 12 pineapple processing industries. The primary data were also derived from self-administered questionnaires prepared by the researcher. In addition, purposive sampling was employed. The information obtained from the interviews provided primary research data that supported the study. Primary data included respondent characteristics and field observation results. Secondary data were collected from agricultural institutions, including the Centre of Data and Agricultural Information Systems, Indonesian Ministry of Agriculture, Agricultural Agency of Riau Province and the Riau Central

Bureau of Statistics, as well as form the annual reports of other agencies relevant to the focus of this study. Secondary data included the number of pineapple processors in Kampar District, the area of pineapple plantations and the number of growers cultivating pineapple.

Data Analysis

The data were analysed via supply chain analysis. The supply chain is covering the full range of activities required to bring products or services through the production phases and delivery processes to consumers, including disposal after use [8]. Supply chains can be mapped and analysed using supply chain analysis, which can include qualitative and/or quantitative tools.

There are no fixed rules on which research approach is better, but there are strong grounds for recommending that a qualitative approach is used first, followed (time and resources permitting) by a quantitative study [9].

Result and Discussion

Patterns in Pineapple Supply Chains

There were three patterns of Pineapple Supply Chain in Riau Province: 1) The Supply Chain for the Traditional Market, 2) The Supply Chain for the Modern Market and 3) The Supply Chain for the Processing Industry. In the traditional supply chain, there were several actors, including farmers, traders and consumers. The most common pattern was farmers-trader-consumers, with actors selling fresh pineapples. In general, farmers harvest their pineapples and transport them to the roadside. Prices are determined based on the quality of the pineapples. Traders have the authority to determine quality by grading the pineapples, including the super grade for large, ripe pineapples.

The most common supply chain in the modern market was farmers- traders- modern market-consumers. The pineapples were sold in the form of fresh pineapples. Farmers sold pineapples to traders, who sold them to supermarkets. Supermarkets bought pineapples at a price per kg instead of per fruit, resulting in a higher price for traders. However, it is important for supermarkets to sort pineapples, to ensure good quality pineapples before selling to customers.

The pineapple supply chain for industrial processing begins with farmers selling pineapples to traders. Traders then sell to the processing industry, which produces pineapple chips and pineapple toffee. All processed pineapple products were sold through retailers, including supermarkets, in Riau Province.

The Roles of Actors in the Supply Chain

Farmers

Farmers in the study area have different characteristics with respect to age, gender, education, farming experience and number of dependents. These differences affect their habits and techniques. Age, education and farming experience greatly affected fresh pineapple productivity.

Young farmers are usually more dynamic, have greater physical ability and the courage to take risks. Older farmers have the farming experience to produce good quality pineapples.

According to the study, the average age of pineapple farmers in Kampar District was between 15 and 55 years old. The respondent’s principal job was as a farmer. The varieties of pineapple cultivated were Cayenne and Queen, with Cayenne the most popular. In general, planting method used by farmers was a monoculture cropping system, with conventional methods still employed.

The stages of the production process included tillage, acquiring seeds, planting, fertilising, plant protection, harvesting and post-harvest. The main inputs used by farmers includes pineapple seed, fertilisers, insecticides, labour and agricultural equipment.

Traders, Processing Industry and Supermarkets

A few farmers sold their pineapples directly to customers along the Bangkinang-Pekanbaru road. Other farmers sold their pineapples to a village collector trader, by taking them to the side of the road. Pineapple prices at the farm level varied, based on the type and grade of the pineapple.

Farmers have many alternative buyers, including traders, the processing industry and the traditional and modern markets. For traders, the main input was fresh pineapples of all varieties.

The system of coordination between farmers and traders was based solely on information submitted by farmers. If farmers sell directly to traders, they have to harvest their own pineapples, and place them on the roadside. At harvest time, farmers tell the traders the pineapples are ready to collect from the roadside. In contrast, selling to merchants who supply fresh pineapples to the wholesale market involves a fixed subscription supply, although this occurred without a written contract.

For the processing industry, the main inputs were fresh pineapples and equipment such as cutting machines, dry vacuum processors to process pineapple chips and packaging equipment.

For supermarkets, the main inputs with respect to pineapple products were fresh pineapple and pineapple chips.

Governance Structure

Method of Coordination between Actors in the Traditional Market Chain

The governance structure in this value chain was informal: there were no binding contracts among actors along the chain and the coordination system between farmers and traders was based on information submitted by farmers.

Method of Coordination between Actors in the Modern Market Chain

Similar to the traditional chain, there were no formal contracts between farmers and traders or between traders and supermarkets. The most common contract was in the form of an informal or oral agreement. There were no long-term contracts between traders and supermarkets: in the harvesting period, pineapples are available in supermarket; in other periods, pineapples are not available in supermarkets or are sold at higher prices. To supply modern markets, the trader follows the following procedures: cuts the pineapple crown – selects good quality pineapples to sell to customers – weighs pineapples, based on customer requirements -washes and wipes the pineapples – packs them using baskets or boxes made of wood – transports pineapples in a truck.

In terms of quantity, the supermarket purchased all pineapples supplied by traders; currently, the volume of pineapples supplied by traders is still below the supermarket's target. Given this, the formation of marketing institutions, such as cooperatives of farmers and traders, which educate on quality standards, could help ensure pineapples meet standards and have been subject to good handling practices. Supermarkets only buy grade A pineapples, bought from farmers by traders at Rp 3,500/fruit, from traders by supermarkets at Rp 7,000/fruit, and from supermarkets by consumers at Rp 10,000/fruit.

Method of Coordination between Actors in the Processing Industry

This supply chain is used for the specific pineapple variety Queen (or Moris), because of its sweet taste, slightly sour flavour and low fibre content. In terms of quality, the pineapples should be well ripe, with a yellow colour. Traders supply such pineapples directly to the processing industry. Prices are determined based on quality, ranging between Rp 2,500-3,000 per fruit for high quality fruit, and about half that for lower quality fruit.

There was no written contract between the manufacturer and the traders; again, the most common contract was in the form of an oral/informal agreement, and dependent on trust and habits. Because the quantity of pineapples still lies below industry requirements, traders are able to sell all the pineapples they have. Traders sell to the processing industry if pineapples are over mature, causing concern about damage during the transportation process. The processing industries produce pineapple chips, which they sell in bulk (5kg) packs. The price of pineapple chips is approximately Rp 80,000-100,000 per kg.

Strategy Formulation to Link Smallholders to the Modern Market

Opportunities and Challenges Selling to Modern Market

There are opportunities to sell to modern markets, with the many modern retail markets (supermarkets, malls and a hypermart) in Pekanbaru, Riau. Some modern retail markets already sell fresh fruit, including pineapples. Large numbers of modern retail markets create new market opportunities for pineapple farmers. The main variety of fresh pineapples sold in modern retail markets was Cayenne, with almost all supermarkets in Pekanbaru selling this variety. Besides being consumed as fresh fruit, pineapples can be processed into pineapple chips and pineapple toffee. The development of the pineapple chip processing industry, together with related outlets in Riau, has provided new market opportunities for pineapple farmers. The development of this industry is evident in the 10 outlets selling pineapple chips or toffee as souvenirs from Pekanbaru.

However, there are also challenges pineapple farmers face in selling to the modern market:

- Quality. To sell their product to supermarkets, farmers must produce pineapples in higher quality compared with those destined for traditional chains. The weight and colour specifications must be met. The main problem in the farmer level is that pineapples have been planted using traditional methods, which can limit their capacity to produce high quality pineapples. Although there is guidance from Riau Agricultural Service Office with respect to pineapple plantations, only a few farmers were consistent applying the technical recommendations, especially on peat soil, because of limitations in cash, capital and labour. Although most supermarkets and the processing industry in Riau are interested in the Queen variety, other varieties are still required and sold.
- Payment and cost system. Supermarkets provide payments to their suppliers fortnightly, or at greater duration, but suppliers, particularly farmers, prefer immediate cash payments. Supermarkets also prefer to buy pineapples that meet their criteria. Given this, pineapple farmers prefer to sell through middlemen, since the middlemen will buy all grades of fruit. Marketing of pineapple chips and pineapple toffee in the modern market also faces several obstacles.
- Supply continuity. Maintaining the supply continuity required by supermarkets, in terms of both quantity and quality, is the main issue for pineapple farmers. Pineapple cultivation was done on a small scale (0.5ha), limiting supply continuity. In addition, the first harvest is available after 12-24 months. Pineapple cultivation is generally applying appropriate cultivation technology and harvesting and post-harvest handling; inappropriate harvest and

post-harvest handling is evident in the appearance and quality of pineapples, which generally do not meet the quality standards of the supermarkets. Maintain supply continuity is also important in the sustainability of the pineapple chip processing industry.

Strategies to Link Small Farmers to the Modern Market

Cultivation, Harvest and Post-Harvest Technology

To connect farmers with the modern market, farmers have to produce pineapples that meet the specifications posed by modern markets. The greatest difficulty in the application of the new technical standards of cultivation, harvest and post-harvest handling of pineapples is farmer risk avoidance. This attitude means farmers are reluctant to invest in new varieties and methods to meet standards of cultivation, harvest and post-harvest handling. Therefore, it is important to recommend technology that does not burden farmers in terms of cost: the least-cost technology for pineapple cultivation is essential. Thus, there is a challenge for researchers at the Indonesian Centre for Horticultural Research and Development to create this kind of technology to be introduced to pineapple farmers.

Currently, one technology to improve quality that can be applied by farmers in Riau is wrapping the fruit using a special plastic or a corrugated carton board. Packaging technology must allow plentiful ventilation, as mould can grow in stagnant air. Thus, packaging technology can reduce disease risk as well as improving the appearance of pineapples. This additional activity is considered by farmers cumbersome and costly. In wrapping activities, farmers incur additional costs in buying wrapping or corrugated carton board, as well as additional labour costs. The challenge is how to provide incentives for farmers to apply these operational standards in pineapple production to produce pineapples that meet the specifications of the modern market.

Increasing the Role of Liaison Organisations (Middlemen)

Liaison Organisation (LO) is a marketing institution, such as Terminal of Agribusiness (TA), Sub-Terminal of Agribusiness (STA) and Farmers' Cooperative. In addition, LO contributes to ensuring the sustainability of the relationship between farmers and modern markets with respect to quality and quantity aspects. The TA is located in the city of Dumai, relatively far from Kampar District. Currently, the performance of TA in Riau is non-optimal because of a lack of cash capital, resulting in payment delays to farmers, who then prefer to sell pineapples to traders instead of the TA.

An important role performed by the TA is sorting pineapples to ensure good quality pineapples are sold to the cooperative, which on-sells both local and regional market. The TA also distribute particular quality of pineapples to traditional chain or to the processing industry. As there were 12 pineapple processors in Kampar District, which then process particular quality of pineapple into pineapple chips and pineapple toffee.

Strengthening the Role of Farmer Groups

The role of farmer groups is important to linking farmers with modern market. To transform farmers from production-oriented to market-oriented, farmer groups must understand marketing.

This paradigm shift will occur more easily through a group than via an individual approach.

Therefore, it is necessary to strengthen farmer groups in Riau. The formation of farmer groups will help farmers to access new knowledge through training, inputs needed for farming, increase the volume of business to achieve an economical scale and improve the bargaining power of farmers [10].

Field observations indicated that the pineapple farmers in Kampar District have been organised into farmer groups that have been progressing well, as demonstrated by the regular meetings held by the farmer groups at least once a month. Meetings sometimes include training provided by the Agricultural Service Office in Riau Province; however, such training has been oriented to production. To be able to participate in the modern market, the farmers need training related to marketing management and contract negotiation.

One of the barriers faced by farmers in Riau in participating in the modern market is the farmers' inability to negotiate and meet the contract specifications required by the modern market.

The existing delayed payment system imposed by supermarkets means farmers prefer to sell pineapples to middlemen, who pay in cash. It is considered more profitable by farmers, turnover is faster. The local government could act as a contract negotiator and facilitator between farmers and supermarkets to produce a win-win solution. To anticipate problems, the local government should help farmers in obtaining flexible contracts that allow for renegotiation when problems arise. The local government could also act as an arbitrator, to resolve problems that arise.

Contract specifications should be directed to the human resource development of farmers.

Farmers must be equipped with knowledge about the background of the contract, the cost of production, and pricing. In addition to ensure farmers produce as required in the contract, then the local government should provide guidance in determining the schedule of farmers planting, cultivation techniques and post-harvest handling.

Conclusion

Actors involved in the pineapple supply chain in Riau include farmers, traders, food processors, cooperatives, modern retail markets and consumers. The governance structure among actors along the chain was mostly in the form of informal contracts; formal contracts only exist between cooperatives and modern retail markets.

The number of modern retail markets and food processors is on an increasing trend in Riau, providing new market opportunities for pineapple farmers to increase production. However, to participate in such chains (modern retail markets and food processors), farmers must face several challenges, including the higher quality requirements imposed by modern markets, payment delays and costs associated with supplying supermarkets and ensuring supply continuity.

There are several strategies that can improve the participation of farmers in the modern market, including applying technical standards to cultivation, harvest and post-harvest technology, increasing the role of liaison organisations (middlemen), and strengthening the role of farmer groups. All previous technologies introduced to improve pineapple quality have implied additional costs for farmers, imposing a burden on farmers. Therefore, urgent efforts should be made by the researchers of the Indonesian Centre for Horticultural Research and Development to create low-cost technologies for producing good quality pineapples, to meet the requirements of the modern market. Local governments should facilitate farmers to gain access to modern markets, by facilitating partnerships between either farmer groups and existing, well-established suppliers or between farmer groups and supermarkets.

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The Pricing System of Shallot at Producer and Consumer Levels

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Abstract

The price of shallot tends to fluctuate every day. The aims of this research are: (1) to analyze factors that affect the price of shallot at producer and consumer level in Indonesia and (2) to analyze trader's behavior on pricing systems of shallot. Vector Error Correction Model (VECM) and Game Theory method are used in this study. The results showed that there was no variable that has significant influence on the price of shallot at consumer level in the short term. The variables that significantly influence shallot price in the long-term were wholesale prices, supply, fuel prices, and wholesale price variables had positive significant effects on price formation at the consumer level. The prices of shallot at retailer was more responsive to the price changes than the price on merchant and wholesaler level. Each economic actor has their own strategies to respond to other economic actors strategies. Based on the results of Game Theory analysis, the pricing strategy by traders showed that every trader in the market applied a collusion strategy by offering the same selling price even though there was no intense communication among traders. The strength of market prices could be reflected in the purchasing power of consumers.

Keywords: game theory, market conduct, price, shallot, Vector Error Correction Model

Introduction

Indonesia is one of the largest countries that produces horticulture products in the world.

Among horticulture products, Shallot is one of the biggest commodities produced in Indonesia.

For the people of Indonesia, shallot is one of the most important vegetable used in various food.

In 2016, Indonesia's total shallot production reached 1.45 million tons. Almost all Indonesians consume vegetables, or around 97.29 percent [1]. The majority of vegetables consumed come from purchases. Only about 27 percent of the rural population consumes vegetables from their own production and only 6.4 percent in urban areas. Considering that the majority of horticultural product consumption comes from purchases, affordability and price stability need to be achieved.

According to National Socio-Economic Survey in [12], household consumption of shallot tend to rise every year.

Price is one indicator to determine the efficiency level of the supply chain for a commodity.

The price of shallot tend to fluctuate overtime. Shallot are one of the commodities contributing to the inflation rate. Prices of shallot in Indonesia experienced relatively high price fluctuations at the consumer level and at the producer level during the period 2011-2017. In that period, prices at the consumer level tended to be more volatile than the producer level. The lowest price of shallot at the farmer level occurred in December 2011 with a value of Rp 12,244.39. While at the consumer level the lowest price occurred in January 2011 with a value of Rp. 14,226.00. The highest price of shallot at the farmer level occurred in January 2017 with a value of Rp. 28,511.44.

While the highest price of shallot at the consumer level occurred in August 2013 with a value of Rp. 36,582.00. Improvement of market information systems and provision of storage facilities are needed to prevent inequality in price formation between producer and retailer in the short term [10].

Demand for shallot will continue to rise along with the increasing number of populations. In 2016, shallot production experienced an increase of 3.93 percent per year, supported by an increase in harvest area of 7.16 percent per year so that productivity rose 1.05 percent per year and experienced a surplus of 997 thousand tons (BPS 2016). [11] and [7] proposed that the formulation and implementation of price stabilization policy require information on price fluctuations and disparities caused by price changes in a market which are transmitted partially to the prices determined by other markets. The aims of this study are: (1) To analyze the factors that influence the price of shallot at the producer and consumer levels in Indonesia; and (2) to analyze the behavior of traders in determining the price of shallot.

Methodology

This study was based on primary and secondary data. Primary data was in the form of traders' behavior in pricing, selling price determination strategies, and marketing agency strategies. The data was obtained by interview using a questionnaire list to the shallot wholesalers and retailers who made purchases at the wholesaler and were considered to represent the study population. The shallot collector trader survey conducted in Brebes Regency and surveys to wholesalers and retailers were conducted at Kramat Jati Central Market in East Jakarta and Caringin Market in Bandung City by the purposive sampling technique.

This study used secondary data in the form of monthly time series data from 2011 to 2017.

Secondary data was obtained from relevant sources such as the Central Statistics Agency (BPS), the Industry and Trade Office (Disperindag) of DKI Jakarta, Agriculture Service for Food Security and Horticulture Office of Brebes Regency, Department of Agriculture and Food Crops of West Java Province, Ministry of Agriculture of the Republic of Indonesia, Kramat Jati Central Market (PIKJ), and other literature relevant to the research. The price of East Jakarta consumers uses an approach with the price of DKI Jakarta. These data was analyzed using E-Views 8 software.

Results

Vector Error Correction Model (VECM) Analysis

To analyze the factors that influence the price of shallots at the producers and consumers level in Indonesia, an econometric model was estimated using the Vector Error Correction Model (VECM) estimation technique. Consumer prices of shallots per kilogram period t (LnCP) in DKI Jakarta are allegedly affected by producer price of shallot per kilogram of t period (LnPP), wholesale prices at Kramat Jati Central Market per kilogram of the t-period (LnWP), Supply of shallot to wholesalers per ton of t-period (LnS), and The Price of Gasoline per liter of the t-period (LnPGS), and also αij is regression coefficient of the VECM model, ε_t is the error term in the t-period, and t is the monthly period from January 2011 to December 2016. The model used in this study was adopted from the Sahara and Wicaksana models in [13]. The VECM restriction model used in analyzing consumer price formation is as follows:

$$\Delta lnCP_t = \alpha_0 + \sum_{i=1}^{1} \alpha_1 lnWP_{t-1} + \sum_{i=1}^{1} \alpha_2 lnPP_{t-1} + \sum_{i=1}^{1} \alpha_3 lnS_{t-1} + \sum_{i=1}^{1} \alpha_4 lnPGS_{t-1} + \gamma ECT_{t-1} + \varepsilon_t$$

Game Theory Analysis

Game theory analysis is used to analyze the behavior of traders in determining the price of shallot. The assumption in this game is common knowledge [13]. Through this approach, the pricing strategy that is carried out at the level of wholesalers and retailers can be seen. The Pareto Optimum is used as an indicator, if there is a change, there will be a loss for all actors.

The first step in the analysis of the game theory in this study is to determine the elements in the game theory used in this study. [5] explains that game theory has 4 elements, including players who have rational choices, decision strategies, payoffs received by each player in every decision they receive, and information to achieve Nash equilibrium conditions. The analysis can be explained in the payoff matrix listed in Table 1.

Table 1. Matrix of pricing strategies by retailers in one market

Economic Actors$_i$2	**Economic Actors$_i$1**	
	Competitive Price	Market Price
Market Price	α_1, α_2	α_2, α_2
Competitive Price	α_1, α_1	α_2, α_1

Note: α_i = payoff strategy

The games carried out by wholesalers with retailers in this study were applied through the Nash procedure. [8] explains that the approach most often used to explain balance in games is Nash equilibrium. In the process of bargaining with consumers, retailers have a strategy to maximize Minimax (highest profit).

Results and Discussion

Factors Affecting Shallot Prices at Producer and Consumer Levels in Indonesia

The VECM test was used to see the effect of producer prices (PP), wholesale prices (WP), supply (S), and the price of gasoline (PGS) on consumer prices (CP) of shallot. Prior to VECM estimation, several tests were carried out on these variables: stationary test, optimum lag test, VAR stability test, and cointegration test.

Stationary test results showed that these variables are stationary in the first difference at a significance level of 5 percent. This indicates the absence of unit roots in each variable used in analyzing the influence of producer prices (PP), wholesale prices (WP), supply (S), and the price of gasoline (PGS) on consumer prices (CP) of shallot.

The minimum *Schwarz Criterion* (SC) information showed that the optimum lag of these variables is in lag 1. This test showed the reaction time of a variable against other variables and can also eliminate autocorrelation problems in the VAR model [4]. While the Vector Autoregressive (VAR) stability test results showed that the VAR system was stable because the modulus value is <1 and located in its unit circle. The results of the cointegration test at 5 percent significance level using the Johannsen cointegration test showed that all variables in this study were integrated in first degree (1). This can be interpreted as a long-run balance between variables.

After being tested that there is cointegration on the model, the next step was the estimation using VECM. The VECM estimation results at the significant level of 5 percent showed the relationships between variables in the short and long term. Based on the results of the analysis, in the short term there are no variables that have a significant effect on the prices shallots at consumer level.

Variables that have a significant effect on long-term estimation were **wholesale prices, supply,** and **gasoline price**. Among these three variables, the wholesale price variable has a significant positive effect on price formation at the consumer level. This is in line with the theory which states that the amount of shallot that will be purchased by traders has an impact on the increase in their business budget (capital) and the trander will increase the selling price to consumers.

Shallot are horticultural crops that have a high economic value, so their role is important in contributing to the economic condition of the country. The price of shallot tends to fluctuate every month; hence the high price of shallots contributes to the inflation rate in Indonesia. Shallot can be sensitive to the expenditure of household needs because it is a daily necessity.

The VECM analysis phase was carried out after unit root testing, VAR stability, optimum lag, and cointegration which showed that there was a long-term relationship between consumer prices, wholesale prices, producer prices, supply and gasoline prices in the model. The significance of each variables is seen through the absolute value of t-statistic which is greater than the value of t-ADF at the real level of five percent which is the same as 1.965, then it can be concluded that the variable has a significant influence. Table 2 shows the results of the VECM estimation on the shallot price model.

Table 2. Results of VECM Estimation on the Shallot Commodity Model

ù Variable	Coefficient	t-statistik
Short Run		
CointEq1	-0.258799	-1.05725
D (LNCP (-1))	-0.222144	-0.89680
D (LNWP (-1))	0.367291	1.14825
D (LNPP (-1))	-0.264258	-1.21173
D (LNS (-1))	-0.228469	-1.87656
D (LNPGS (-1))	-0.265390	-0.52851
Long Run		
LNCP (-1)	**-0.948868**	**-6.31031**
LNPP (-1)	-0.076574	-0.58654
LNS (-1)	**-0.279094**	**-3.78637**
LNPGS (-1)	**0.225827**	**2.15132**

*Note: * Significant at five percent real level*

The VECM estimation results in Table 2 proves that there are no variables with significant influence on the consumer prices of shallot in the short term. Variables that have a significant effect on long-term estimates are wholesale prices, supply, and gasoline prices. Among these three variables, the wholesale price variable has a significant positive effect on shallot price at the consumer level. This is consistent with the results of the research by [6] which states that the first information obtained by retailers in offering selling prices to consumers comes from wholesalers so that the response in price is faster compared to other factors.

Shallot supply variable also has a positive influence to shallot price at consumer level and the gasoline price variable negatively affects the retail price of shallot. The influence of shallot supply result is different from the theory, but through the results of interviews with traders, shallot is a very responsive commodity, in contrast to garlic which can last for a long time.

The gasoline price variable used is the price of "Premium" type by Pertamina and not all traders use "Premium" type as a proxy for transportation costs from the wholesale market to the retail market. So, the increase in premium gasoline prices does not directly affect the retail price of shallot. [2] support that disruption or natural disaster during the distribution period in this case is more influential than changes in gasoline costs and supply does not have a significant influence on price formation at the consumer level. [3]

Also explained that there is no long-term relationship between the West Java producer area and Kramat Jati Central Market wholesaler, so that market efficiency has not been achieved.

Therefore, a significant variable in the formation of the price of shallot are the wholesale price, supply and price of gasoline in the long run.

Based on the results of The Impulse Response Function (IRF) analysis with the identification based on the Generalized Decomposition, it can be concluded that the price of shallot at the consumer level responds positively but in-significant impulses from producer prices from the beginning to the end of the period. The response of the consumer price of shallot to producer price shocks was 0.178468 in the second period. Each increase in the price of shallot producers by one standard deviation will increase the level of consumer price of shallots by no significant amount of 0.178468 percent. Long run balance occurs in the 23rd period of 0.163877 percent. This result is supported by previous studies conducted by [9] and [15] which found that price information also determines price integration and transmission that occurs.

Beside that, synergy between short-term policies in the form of tax and customs incentives, medium-term policies in the form of increasing agricultural productivity and long-term policies in the form of adaptation and mitigation to climate change can create price stability and adequate supply of agricultural products to ensure people's welfare [14].

The Game Theory of Shallot Pricing

Market prices are prices that generally apply to a market, and competitive prices are prices lower than market prices in response to the current market prices. The application of competitive prices aims to attract consumers. Every economic actor has each strategy to respond to the strategies of other economic actors. Market prices apply when prices are generally increasing so that prices are generally applied evenly with the margins received from previous economic actors.

Table 3 describes the pricing strategies carried out by wholesalers for shallot. Nominal Rp.12,000 per kg is a competitive pricing strategy for shallot collecttor trader in response to a higher market price of Rp. 17,500 per kg in 2017.

Table 3. The game theory matrix between collect trader in Kramat Jati Central Market, 2017

Collector Trader 2	**Collector Trader 1**	
	Competitive price	**Market price**
Market price	12 000, 17 500	**17 500, 17 500***
Competitive price	12 000, 12 000	17 500, 12 000

*Description: * Nash equilibrium*

The choice of wholesaler strategies also consists of competitive pricing strategies and market price strategies which can be seen in Table 4. The price of Rp 16,000 per kg for shallot in 2017 becomes a competitive pricing strategy applied by wholesaler to attract retailer to buy shallot from them. The market price of shallot at wholesaler level was IDR 45,000 per kg. The high market prices were responded by collectors with lower competitive prices so that prices did not continue to increase.

Table 4. The game theory matrix between wholesalers in Kramat Jati Central Market, 2017

Wholesaler 2	**Wholesaler 1**	
	Competitive price	**Market price**
Market price	16 000, 45 000	**45 000, 45 000***
Competitive price	16 000, 16 000	45 000, 16 000

*Description: * Nash equilibrium*

Retail traders are more responsive to price changes, especially for shallot because the price is always fluctuating every month. At the level of retail traders, the prices applied are higher than the prices of collector traders and wholesalers. Competitive prices as a response to the implementation of higher market prices for shallots ranged from Rp. 22,000 per kg for the lowest selling price. In general, the market price in 2017 for shallots is around Rp. 50,000 per kg. Shallot are perishable, so the selling price is unstable.

Table 5. The game theory matrix between retail in Kramat Jati Central Market, 2017

Retailers 2	**Retailers 1**	
	Competitive price	**Market price**
Market price	22 000, 50 000	**50 000, 50 000***
Competitive price	22 000, 22 000	50 000, 22 000

*Description: * Nash equilibrium*

Conclusion and Recommendation

Based on the research results, the conclusions derived were as follows: The wholesale prices are the variables that most influence consumer prices of shallot, so various efforts must be made to protect consumers. Market price (collusion) becomes the preferred strategy adopted by retail to maintain profits. So in the retail market, consumers paying a higher prices.

The government can implement the ceiling price policy. Ceiling price policy at the consumer level expected to avoid exploitation behavior by trader's intermediary, in the form of determining the price of shallot that are too high at consumer level.

Another policy is that the government needs to determine the reasonable price range, which is a price level that is not exploitative for consumers but still provides the ideal margin for the traders.

Optimization of the supervision function through the Business Competition Supervisory Commission (KPPU).

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Potential Development of Organic Horticulture Oriented to Export Market in Bintan Border Area

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Abstract

Bintan regency has promising horticultural potential. Many varieties of good vegetables and fruits have not been optimally cultivated. However, some vegetable and fruit seeds such as zallaca, guava, mango, shallots, vegetables, corn, watermelon, and others have initially been developed.

The objective of this study is to obtain the most appropriate strategy in order to penetrate the export market for horticulture (organic vegetables and fruits) to Singapore. There are four strategies to penetrate the Singapore market i.e.: (a). SO, Strategy, ST strategy, WO strategy and WT strategy. Key factor of success position of this research results is in the quadrant I, which means that the most appropriate strategy used for the development and marketing of organic vegetables/fruits for export in Bintan Island is the S-O strategy, namely (i) follow up on the commitment of the top leadership of the Ministry of Agriculture to take a chance the negotiation forum in the Special Economic Zone (SEZ). (ii) Implementing Innovation of organic farming recommended by IAARD (liming, balanced fertilizers, bio-fertilizer, bio-control of pest, light traps) to take high demand of organic vegetables and fruits for Singaporeans society through improving the quality of organic products produced especially efforts to maintain freshness and guarantee of food security through the application of HACCP. This innovation support is directed to develop superior commodities in an industrial and sustainable agribusiness system based on local resources.

Keywords: Bintan, border area, potential market, horticulture, domestic and export

Introduction

Development of horticulture commodities (vegetables and fruits) in the border region of Bintan should be projected on the existing activities and businesses and needs to be supported from the local government. The development horticultural commodities should also be efficient, not polluting the environment and culturally acceptable (Menkhoff *et al*., 2007; Chay *et al*., 2007; Yannopoulos, 2011; Noor *et al*., 2012; Norazah Mohd Suki, 2015; Barbarossa C. and De Pelsmacker, 2016; Soegoto, 2018).

From the data of land availability, it appears that vegetables and fruits have a good potential to be developed in Bintan Regency. The widest cultivated land area is in the sub-district of Teluk Sebong (3.275 ha), followed by Toapaya sub-district (2.000 ha), Bintan Timur (791 ha) and Gunung Kijang (555 ha). The area of potential land is 14,841 ha, and which has been cultivated 8,098 ha (56.5%). The cultivated commodities are dominated by vegetables (4,335 ha), followed by fruits (3,119 ha), secondary crops (641 ha) and rice (3 ha) (Bappeda Bintan, 2016).

Bintan region is in a free trade zone (FTZ). The distance or mileage (using Ferri ships from Bintan to Singapore BBT International (Lagoi) – Tanah Merah Port (Singapore) takes 55 minutes.

In 2009, Indonesia is only able to supply 6% of Singapore's fruit and vegetable needs. Indonesia ever controlled 30% of the market share of Singapore fruits and vegetables. The main problem in fulfilling export horticultural commodities to Singapore is the availability of bulk quality superior seeds. Technology exists but commercialization is still lacking (Amalia, 2016). Nevertheless, Indonesian fruits and vegetables are still in demand in foreign markets. This is demonstrated by the export data which shows that exports of 10 vegetable commodities from January to September 2015 increased by 32%. The same holds true for the exports of nine fruit commodities which grew by 29.8% during the same period.

According to the Ministry of Agriculture, the five largest horticultural product exports in 2015 were pineapples, mangosteen, cabbage, chillies and ginger. The available market share per shipment includes: mustard oil (1,800 kg), Green Mustard (1,800 kg), Curry Mustard (450 kg), White Mustard (450 kg), Kangkong (500 kg) and Spinach (500 kg), cucumber (1200 kg), pariah (500 kg), long beans (1,200 kg), squash (500 kg), eggplant (300 kg), slime beans (200 kg) (Rusdianto, 2012).

Rejection in particular of the above vegetable products in Singaporean market is most likely due to the pesticide residue threshold exceeding the criteria set by the Codex standard and Singapore or AVA authority. Class of organophosphate insecticide residues found in many types of vegetables, such as shallots with a concentration of 1.167 to 0.565 ppm, potatoes contains 0.125 to 4.333 ppm; peppers and carrots contain profenos 0.11 mg/kg, detakmetrin of 7.73 mg/kg, chlorpyriphos 2.18 mg/kg, tulubenzuron 2.89 mg/kg, and permethrin 1.80 mg/kg, respectively.

Pesticide maximum residual limit (MRL) is the maximum concentration of pesticide residues that are legally permitted or known as acceptable concentrations of agricultural products expressed in milligrams of pesticide residues per kilogram of agricultural produce (Ginting and Daroesman, 1982; Shizuka *et al*., 2016; Codex Alimentarius, 2018).

Another problem encountered by the farmers in Bintan island in producing organic vegetables and fruits is less soil fertility and high temperature (280-300C) which is not suitable to develop vegetables/fruits (Afandi *et al*., 2015). Bintan Island is dominated by red yellow podzolic land with an average pH of 5. Until now, the Bintan region has been declared as an arid area, which is mostly former bouxit mining.

The objective of this study is to get the most appropriate strategy in order to develop vegetable and fruit to penetrate the export market (organic vegetables and fruits) to Singaporean market.

Material and Method

The research was conducted at Bintan District, Riau Islands Province 2017. Research was conducted with a quantitative SWOT approach (Hidayat dan Rachmat, 2018). The choice of this method is intended so that the strengths and weaknesses in the development of organic vegetables in the Bintan Islands Riau region can be explored appropriately and measurably by taking into account the opportunities and threats that apply. Data Collection Method is carried out using the following step: (i) FGD (Focus Group Discussion) which are intended to explore information both in the form of primary data and key factor to solve the problem, and (ii) Literature Study (Library Research), which is intended to obtain secondary data originating from existing literature, such as textbooks, reports/documents, information and data from the internet, relevant laws and regulations.

The stages of analysis using the SWOT method quantitatively (Ramadhan dan Sofiyah, 2013; Amalia, 2016; Hidayat *et al*., 2018) are as follows:

a. Compile the Internal Factor Matrix (IFE) and the External Factor Matrix (EFE) which consists of the weight column, rating, and total value which is the result of the weight and rating. The number of internal factor weights (strengths and weaknesses) is one. Likewise, the number of weights of external factors (opportunities and threats) is one. The weight value for each parameter is determined based on the level of urgency of each factor of strength, weakness, opportunity and threat that occurs. This means that the evaluation of one parameter is related to the level of importance of the other parameters. The rating range (rate) is from the lowest 1 to the highest 4.
b. Plotting in a SWOT diagram. This diagram illustrates the position of strength minus weakness (internal) and the position of opportunities minus (external) threats.
c. Develop strategies that are in accordance with the SWOT diagram.

Results and Discussions

The results of weighting internal factors (attachment 1), two big factors of the commitment of the leadership of Ministry of Agriculture (Mentan) with BF of 27%, and Lack of soil fertility (20%). From the results of weighting external factors, the most likely factor from Bintan is the Special Economic Zone (SEZ) with BF of 27% and the high demand for organic vegetables and fruits and Competitors of Chinese and Malaysian Organic Vegetables and Fruits (20%).

Analysis of internal and external factor and the value of support and linkages

The difference between the linkage value (NK) and the Fund rating (ND) is that the linkage value is a synergy value between the factors that make up each factor. In the attachment 1, the value of the largest number is 5 and the lowest is 1. By using the back-value table (ND), all factors that are high or can be interpreted have a strong enough influence. The strongest influence on level 5. The lowest ND is on 3 factors with a score of 2, namely: Limited farmers who produce organic vegetables and fruits, low competitiveness of Bintan vegetables and fruits, and climate and non-tariff barriers can be reviewed again.

By looking at the results of the Relative Relation Value (NRK) that juxtaposes all factors, it can be said that all factors have interrelationships that are close together. This can be seen from NRK for each factor that has a grant-average of 2.00 s.d. 3.45. Thus, all existing factors have a significant influence on the performance of organic horticulture exports in the Ministry of Agriculture.

To determine key factors, Total Weight Value (TNB) is needed from each factor by nominating the Support Weight Value (NBD) with the Linkage Weight Value (NBK).

From attachment 1, the internal and external reports can also find out the ordinate position of the organization (in the square of S¬W and O¬T). Where the position of internal and external factors is: Internal: Strenght (+) = 3.78, Weakness (¬) = 2.55, Posisi Ordinat (+) 1.23, Exsternal: Opportunity (+) = 3.48, Threats (¬) = 2.05, Posisi Ordinat (+) = 1.43.

Map of strength position

Organizational coordinates (Cement) at SW are + 1.23 and Organizational Coordinates on OT are + 1.43, meaning that they are in Quadrant I or SO strategy or, meaning the strategy is more towards expansion/development/growth. SO, Strategy, use the strength to take advantage of opportunities:

1. Follow up on the commitment of the top leadership of the Ministry of Agriculture to use the negotiation forum in the Special Economic Zone (SEZ).
   The opportunity to export Bintan vegetables and fruit to Singapore is wide open. The current market share is less than 10 percent. To meet these targets, continuous production in quality, regular quantity, application of good agricultural practices, food security, and adequate supply chains are needed. To realize an export contract with overseas (Singapore), exporters in Bintan need to partner with vegetable and fruit farmers (Helmstadter, 2003, Hutching and Michailova, 2004). On farm, it is necessary to support and guide farmers to improve product quality. Indonesia's exports in 2009 were around 32,000 tons or around 6.5 percent. The export market target for 2014 – up to now (30 percent), is equivalent to 130,000-135,000 tons. Horticultural commodities that are potentially exported such as potato, petsai (Chinese cabbage), beans, tomatoes, long purple eggplants, pineapples, mangosteen.
2. Use of Innovation of vegetable and fruit farming recommended by IAARD to take demand of vegetables and fruits for Singaporeans society.
   Innovation is a broad concept from a new marketing method or a new organisational method in business practice. Innovation, focusing on how to put ideas into practice and how this technology applied to organic farming. Innovation in the organic food and farming sector depends on the functioning of the system as a whole and this systems perspective is becoming more widespread in designing innovation support. Several technology Innovation has been resulted by IAARD such as Creating Green Consumer of vegetable (Noor *et al*., 2012); Yellow Sticky trap on hot pepper (Gunaeni *et al*., 2014), repellency of essential oils (Hasyim *et al*., 2014); rainshelter and biopeticide on Chili (Setiawati *et al*., 2018); Low external input technology for chilli (Setiawati *et al*., 2018) and other institution such as trapping paper media (Hakim *et al*., 2016). To improve low soil fertility in Bintan, some organic fertilizer such as stable manure, compost, liming, balanced fertilizer can be utilized (Novizan, 2002; Suntoro, 2003).

Conclusions and Recommendations

Conclusions

SWOT analysis can be used in pre-crisis planning and prevention of management of crisis.

Position of Key Success factors (FKK) is in quadrant I. The most appropriate strategy used for the development and marketing of organic vegetables/fruits in Bintan Island is the S-O strategy, namely (i) follow up on the commitment of the top leadership of the Ministry of Agriculture to use the negotiation forum in the Special Economic Zone (SEZ), (ii) Use of Innovation of organic farming recommended by IAARD (liming, balanced fertilizers, biofertilizer, bio-control of pest, light traps, to take high demand of organic vegetables and fruits for Singaporeans society through improving the quality of organic products.

Recommendations

With this SWOT study, it is expected to provide an overview of the stages of formulation of objectives starting from the vision and mission that produces values. The vision, mission and values are simultaneously analysed by considering environmental factors that influence both the internal environment, namely the external environment.

Ministry of Agriculture must conduct market research regularly to be able to find out changes in consumer behaviour towards the quality attributes of fresh vegetables. Monitoring residual content in fresh vegetables by applying HACCP from the civilization process to the distribution process.

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Evaluation of Internal and External Factor of Organic Vegetable and Fruit Exports from Bintan to Singapore (Bintan, 2017)

No	Internal and Eksternal Factors	BF (%)	ND	NBD	1	2	3	4	5	6	7	8	9	10	11	12	NRK	NBK	TNB	FKK
	Internal Factors																			
	Strenght (+)																			
1	Commitment of the Minister of Agriculture	27%	4	1,08		5	5	4	3	4	3	3	4	2	3	1	3,36	0,91	1,99	
2	Standardization of GAP, Audit and Government regulations	13%	4	0,52	5		2	2	4	2	4	5	0	3	3	4	3,09	0,4	0,92	
3	Availability of Vegetable and Fruit Organic Technology	13%	4	0,52	5	2		4	2	2	2	4	1	2	3	3	2,73	0,35	0,87	
																			3,78	
	Weakness (-)																			
4	Limited Skilled Farmers Who Cultivate Organic Vegetables and Fruit Crops	13%	3	0,39	4	2	4		4	2	2	2	2	4	2	4	2,91	0,38	0,77	
5	Low Vegetable and Fruit Competitiveness	13%	2	0,26	3	4	2	4		4	4	4	2	3	4	4	3,45	0,45	0,71	
6	Less Soil Fertility	20%	3	0,6	4	2	2	2	4		1	2	1	1	3	4	2,36	0,47	1,07	
																			2,55	
	External Factors																			
	Opportunity (+)																			
7	Bintan is a Special Economic Zone	27%	4	1,08	3	4	2	2	4	1		3	4	2	1	1	2,45	0,66	1,74	
8	High Demain of Organic Vegetables and Fruits	20%	3	0,6	3	5	4	2	4	2	3		3	3	2	3	3,09	0,62	1,22	
9	Geographical proximity of Bintan and Singapore	13%	2	0,26	4	0	1	2	2	1	4	3		2	2	1	2	0,26	0,52	
																			3,48	
	Threats (-)																			
10	Competitors of Chinese and Malaysian Organic Vegetables and Fruits	20%	3	0,6	2	3	2	4	3	1	2	3	2		3	4	2,64	0,53	1,13	
11	Global Climate Change	7%	2	0,14	3	3	3	2	4	3	1	2	2	3		1	2,45	0,17	0,31	
12	Non-Tariff Barrier (Asian GAP and MRL)	13%	2	0,26	1	4	3	4	4	4	1	3	1	4	1		2,73	0,35	0,61	
																			2,05	

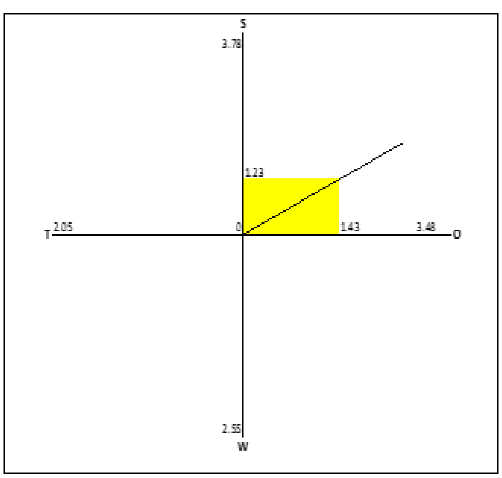

Fig. 1.
Strategic MAP of Ministry of Agriculture Export of Organic Vegetable and Fruit Crops.

Adapting to Changing Trends in Tropical Fruit Production, Consumption and Markets in SE Asia

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Abstract

Total global tropical fruit production is estimated at 92 million tones (FAO). The main tropical fruit producing regions are Asia and the Pacific, which produce an estimated 55 percent of total production while Latin America and the Caribbean produce 32 percent and Africa about 11 percent. Many tropical fruits are indigenous to the Asian region, where they are developed, produced, consumed and traded. According to Food and Agricultural Organization (FAO), in 2016, the major producers of tropical fruits in South East Asia countries produced a total of 48,658,951 tones with Philippines producing 32 percent followed by Indonesia, Thailand and Vietnam at 30, 19 and 9 percent respectively. A recent study by FAO noted that globally only a tenth of minor tropical fruits are exported, thus, opening up potential markets for fruits such as rambutan, mangosteen, dragon fruit, passionfruit, jackfruit, and durian. The outlook of the tropical fruit industry is positive and expected to grow with increasing income levels resulting in changing trends in consumer preference for exotic taste, safe, healthy and quality food. These go in tandem with the robust growth in the tourism industry, emergence of new markets in China and the Middle East, modern retail outlets, e-commerce platforms and increase in regional trade pacts and bilateral arrangements. Some common issues prevalent are lack of postharvest and quality management, frequent pests and diseases incidences, dependence on traditional market systems, lack of market access and limited inclusivity of smallholders. The use of marketable cultivars, improved infrastructure and logistics, emphasis on research and development and new technologies, institutionalization of smallholders, mitigating postharvest losses, increased vigilance and better management of pests and diseases, strengthening SPS regulations and value chain analysis are some measures employed. This paper discusses some of the main initiatives required to adapt to the changing trends in tropical fruit development.

Keywords: consumer preference, production technologies and management, market access, inclusivity

Introduction

The main tropical fruit producing regions are Asia and the Pacific, which produce an estimated 55 percent of total production while Latin America and the Caribbean produce 32 percent and Africa about 11 percent. Many tropical fruits are indigenous and agro-climatically suitable in the South East Asian region, where they are developed, produced, consumed and traded. According to the Food and Agricultural Organisation (FAO), in 2016, the major producers of tropical fruits in South East Asia were Philippines, Indonesia, Thailand, Vietnam, Malaysia and Myanmar producing a total of 15,927,409, 14,922,907, 9,420,051, 4,822,593, 1,136,369 and 1,116,419 tonnes respectively. The major exporters in 2016, were Philippines, Thailand, Vietnam, Malaysia, Myanmar and Indonesia, exporting 1,986,698, 633,462, 229,983, 165,975, 62,028 and 56,935 tonnes respectively. In the same year, the export value for Philippines, Thailand, Malaysia, Vietnam, Indonesia and Myanmar were 916,031,000.00, 689,092,000.00, 74,634,000.00, 49,772,000.00, 33,681,000.00 and 9,669,000.00 US Dollars, respectively (Table 1).

Table 1. Major production and export of tropical fruit in South East Asia in 2016 (FAO)

Country	Production (tonnes)	Export (tonnes)	Export value (USD)
Brunei	2,142	0	0
Cambodia	235,469	376	872,000
Indonesia	14,922,907	56,935	33,681,000
Lao	1,060,090	21,525	4,890,000
Malaysia	1,136,369	165,975	74,634,000
Myanmar	1,116,419	62,028	9,669,000
Philippines	15,927,409	1,986,698	916,031,000
Singapore	0	1,137	3,235,000
Thailand	9,420,051	633,462	689,092,000
Timor Leste	15,502	669	9,000
Vietnam	4,822,593	229,983	49,772,000

(FAOstat, 2017)

Methodology

This paper is written with inputs from the following sources:
1. Desk reviews
2. Field studies and projects conducted by the International Tropical Fruits Network (TFNet)
3. On ground interviews and feedback from industry players
4. Presentations from symposiums, conferences and workshops organised and attended by the International Tropical Fruits Network (TFNet)

Results and Discussion

Current Trends

According to FAO, the tropical fruit industry is expected to grow with the increase in demand for the commodity. The demand is market driven and influenced by the following trends:

a. Increase in income and the willingness to pay for premium produce,
b. Evolution of consumer preference to good quality, safe, healthy, novel, tasty and convenient food,
c. Introduction to new exotic fruit types due to robust growth in the tourism industry,
d. Ubiquitous and convenient retail outlets such as supermarkets, and convenience stores,
e. Efficient distribution and delivery systems due to vast improvements in logistical network, communications and a flexible and interconnected marketplace,
f. Online marketing and e-commerce platforms,
g. Development of technologies along the value chain especially storage technologies,
h. New and emerging markets in China, and the Middle East, strengthening and establishment of regional trade pacts,
i. Compliance to Good Agricultural Practice (GAP) standards and biosafety protocols as prerequisites for gaining market access into importing countries.

Generally, the trends are indicative of a growing consumer base that is now more discerning, adventurous and partial towards new ‘exotics’ and improved fruit types and cultivars that possess high nutritional values combined with health benefits, good taste, freshness and of premium quality. This is also reflective of the production technologies and practices that have to be used and even adapted to sustain production, increase production quantum to meet rising demands and expand the market.

Production techniques and technologies for tropical fruits have improved in the last decade through investments in research and development, resulting in increased productivity for most commercial fruit types. Notwithstanding this optimism, issues and challenges in adapting to these are many, and specific to each producing country, largely attributed to different geographical locations, infrastructural development, climate related shortcomings, state of agricultural development and existing policies. Production related issues include use of unsuitable cultivars, lack of technical know-how, pests and diseases incidences and rising costs in inputs. The issues affect both the big players and smallholders, however empirical findings indicate that the latter is more affected due to lack of capital, limited knowledge and access to modern production techniques, capacity building, financial constraints and limited access to markets.

Quality and Adapting to Market Trends

As in other horticultural crops, the market for tropical fruits is influenced by the different market channels, players along the value chain, consumer preferences, demand and supply and market access. Ultimately, it is the market which decides and dictates the production quantity and quality requirements of the produce. A typical modern consumer market is generally segmented whereby products are produced to cater for the demands and needs of a specific market. Market segmentation contributes to the demand for certain grades according to consumer preferences at the various points of sale, along the chain. To adapt to the new trends, market segmentation for high quality produce at all levels along the chain should be targeted. Markets which demand for high quality fresh produce calls for the growing suitable cultivars, application of recent modern technologies, and promotion of good farm practices to reduce postharvest losses and improve pests and disease management. A traditional market system, with many players along the chain, gives less flexibility to the producers.

Gaining market access for export is another hurdle for many tropical fruit producing countries.

Countries such as Australia, USA and Japan practice stringent import regulations including assessment of production practices, quality and safety standards and sanitary and phytosanitary requirements which producing countries should endeavour to comply with.

Central to adapting to these trends is the key effort to fulfil market requirements with premium quality and safe produce both for the domestic and export markets. This would require efforts to improve productivity through good farm practices and adoption of new technologies in the various production process, including improved cultivars, reducing postharvest losses, better pests and diseases management and quality management.

Suitable New Cultivars and Appropriate and Improved Technologies

Criteria of suitable cultivars should include adaptability to local growing conditions, consistent high yields, tolerance to pests and diseases and possess good market value. Most of the popular and marketable cultivars have been developed by big companies, advanced farmers or multinationals. These varieties have been dominant in the market for a long time, and demand has been very consistent. This includes the domination from MD2 pineapples, whose demand has been increasing since it was introduced in Costa Rica in 1996. Since then the variety has expanded to other countries. Philippines is one of those growing this variety that is exported to other Asian countries. Besides the MD2, the MG3 or 'sweetio' pineapple is also gaining popularity. Another example is the popular durian cultivar such as D200 and D197 (*musang king*), besides the ever-popular Thai '*Monthong*' variety. Planting materials for some of these cultivars are not always available. For bananas, the *Cavendish* is the most developed and highly favoured globally.

There are many recommended cultivars for mango, since is it widely grown in tropical and subtropical environments. The popular ones are the *Nam Dok Mai*, *Carabau*, *Arum Manis*, *Alphons*o and *Tommy Atkins*. Similarly, there are many popular cultivars, both developed and introduced, for rambutan, jackfruit, pomelos, guavas, rose apple, sapodilla and dragon fruit that are suited for the domestic and export markets.

The performance of introduced cultivars should also be evaluated at the different agro-climatic regimes, before they can be commercially pursued. This requires strong research and development with the involvement of advanced growers. Local cultivars can also be developed into a potential commercial crop through selection, breeding and good farm practices. It is thus imperative for producing countries to have good fruit breeding programmes.

Appropriate and Improved Technologies

The lack of technical knowledge in production technology is another setback faced by producers, ostensibly due to lack of research and development initiatives, inability to disseminate research findings to the field for adoption or considered a low priority fruit type for development.

Technical know-how includes the incorporation of good farm practices such as nursery preparation, field growing techniques, precision management in areas of proper fertilizer application and requirement determination, suitable irrigation techniques and water management.

Others include appropriate pruning techniques, judicious chemical use and correct harvesting method and on-field packing. Farm technologies can be effectively extended to growers by capacity building programmes, supported by targeted research and development programmes.

Improved technologies should also cover the areas of postharvest and storage.

Improvements in Postharvest Management

Postharvest management is an important feature in any fresh produce initiative to maintain quality, reduce losses and prolong shelf life. However, implementation, especially in handling, is still lacking. It has been cited in reports that an average of 30 percent postharvest losses occurs for horticultural crops such as fruits. This has been attributed to lack of infrastructure such as cold storage and logistics, the gaps in the value chain, traditional market systems, distance to formal markets, lack of quality requirements and inconsistent demand for the fruit type. The longer the chain and more players involved, the more postharvest losses occur, because handling is done at every point of sale from farm collection to wholesalers to retailers and exporters. Farm packed produce can mitigate this problem; however, this requires low cost alternatives and a direct marketing arrangement with the buyers. In addition to this, the lack of cold chain facilities affects freshness and shelf life, especially if the fruits are destined for faraway destinations.

While postharvest management sometimes is linked to market segmentation, where the produce is sold for specific market requirements, perhaps it should be emphasized that maintaining quality and reducing losses should remain the ultimate goal for all marketing efforts.

Improvements Pests and Disease Management

The constant and sometimes unpredictable threat of various pests and diseases has a major impact on the production of tropical fruits in the region. Fruit growing areas in the humid tropics are more susceptible to diseases than those grown in the subtropical zones because of high temperatures and humidity. One common example is the threat by Fusarium FOC TR4 which causes the banana wilt disease that has devastated production areas in the region, and continues to be a major threat to other banana growing countries. Recently, there has also been reports of the emergence of the banana blood disease and Moko disease on cooking bananas. Other common diseases that have plagued the industry are the papaya ring spot virus, citrus greening disease or Huanglongbing (HLB), and recently bacterial wilt on papayas and canker on pitaya.

To adapt, a holistic approach incorporating biological, chemical, surveillance, monitoring and a robust biosecurity outfit is imperative in managing the problem of pests and diseases. This has to be supported by capacity building programmes to train growers on identification, diagnosis and management of pests and diseases. Major diseases can also be dealt with by the involvement of companies in research or joint research between the public and private sector. Emphasis must be given to improve biosecurity measures because a slack in this area would exacerbate spread of pests and diseases. A concerted effort of both the private and public sectors in conducting research and development should be encouraged. Perhaps a mixed cropping planting system involving more than a one fruit tree crop could be promoted to minimise losses, as compared to monocropping. An area-wide pest and disease management strategy is also recommended when an outbreak is reported.

Public-Private Sector Initiatives

Inclusivity of smallholders to participate actively in the upstream and midstream segments of the value chain of tropical fruits seems to be lacking in many countries in South East Asia. It is becoming more evident that the industry is run by big corporations, companies and those with sound financial capacities. Supply chains in South East Asia are often dominated by the big players who act also as the ‘influencers’ to the industry. This scenario can be improved if these big players work in tandem to assist smaller producers by channelling resources for technical skill-upgrades, adoption of technologies and gaining market or export opportunities. Governments can assist by implementing policies that will institutionalize smallholders into groups or cooperatives.

Policies to Improve Quality and Production

Governments have to put in place policies that would encourage growth of the industry, especially with initiatives related to the production of high quality, export oriented fresh produce.

This would include providing good infrastructure and logistics, supporting research and development initiatives, assist in the promotion and expedite bilateral or agreements with importing countries. Other than this, inclusivity of smallholders by instituting farmers’ groups, encouraging public private partnerships, facilitating capacity building programmes on new technologies and providing low interest financial loans for expansion should be encouraged.

Conclusion

The changing trends of eating habits and food preferences are results of increasing incomes, health consciousness, increasing lifestyle sophistication and extensive travel opportunities. For tropical fruits consumption, modern consumer preferences have shifted towards premium grades that hold high value in terms of quality, freshness, safety, taste and exoticness, in addition to convenience. These trends have created great interest for developing tropical fruits into the mainstream fruit market, especially with the new-found appreciation for minor tropical fruits such as durian, rambutan, pitaya, passionfruit, jackfruit, long an and pomelo. The tropical fruit industry will only be ready to meet the demands of the future through the adaptability of all industry players to these emerging trends, with the support of better distribution connectivity and logistics, introduction of new production and storage technologies and with e-commerce becoming commonplace. Hence, efforts should be enhanced for the use of marketable cultivars, improving infrastructure and logistics, increasing robust research and development initiatives and application of new technologies, institutionalisation of smallholders, mitigating postharvest losses, increase vigilance and better management of pests and diseases and strengthening SPS regulations. The involvement of the private sector with facilitation by national governments and universities especially in research and development, export market promotion, capacity development and institutionalising smallholders are necessary for the tropical fruit industry to thrive and remain sustainable.

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Barrier Factors for Smallholder Farmers in Exporting Mangosteen (*Garcinia Mongostana*, L) from Indonesia to Global Market

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Abstract

Mangosteen is high in demand for export. It gave the largest contribution to foreign exchange.

Even though mangosteen have the potential market for export, but it has many barriers. One of the barriers is from smallholder farmers who produce mangosteen. The objectives of this research are 1) to identify the export barriers that prevent Indonesian mangosteen exportation to the global market in smallholder farmers scale, and 2) to identify the solutions in facing these barriers so that Indonesia can continuously provide mangosteen products to global markets. This research essay used strategic literature review that analyses the export barriers to export mangosteen from Indonesia to global market based on the literatures review on categories of export barriers.

Smallholder farmers faced export barriers such as: 1) the knowledge barrier because lack of implementation of innovative technology such as pre harvest, harvest and post-harvest handling and Good Agriculture Practices (GAP); 2) the resource barrier including lack of capital, production input, and human resources, decreasing productivity and insufficient production; 3) the procedure barrier including lack of infrastructures, high transportation cost, standardization and phytosanitary issues; and 4) the exogenous barrier including climate change, seasonality, geographical location and lack of sense of belonging to the mangosteen commodity. The solutions in facing these barriers are by improving dissemination of technology innovation, improving infrastructure, expanding the mangosteen area and supporting agriculture production, increasing sense of belonging of mangosteen, strengthening smallholder farmer's institutions by vertical integration, and providing leasing for mangosteen cultivation.

Keywords: export barriers, mangosteen, farmers

Introduction

Globalization offers opportunities to increase agricultural exports. The capacity of smallholder producers in developing countries to respond to opportunities of globalization and to take advantage of the opportunities offered by globalization is most concern [1]. Export success appears to be partly based on producers' ability to move into higher quality of their products which gives impact on higher price segments [2]. Sanitary and Phytosanitary (SPS) standards act as non-tariff barrier but these standards could manage by producers to allow them to meet certain standards of SPS in order to capture significant mark-ups price [1]. Many of Indonesia's horticultural products are being rejected by the importing countries because of small defects that occurred during the process of distribution. For example, Indonesia's mangosteen has been banned from entering China in 2010. In 2012, China rejected mangosteen shipments again due to fruit flies [3]. Therefore, the Indonesian Association of Exporters of Vegetables and Fruits (AESBI) is proposed to establish a special area for the cultivation of fruits and vegetables products in the country, with an adequate supervision and distribution [4]. The growing potential of the mangosteen market is both an opportunity and a challenge for national mangosteen producers. The potential market becomes an opportunity when follows by increased production and quality of fruit. On the other hand, it would be a threat if the national mangosteen production is not able to compete in the domestic and markets. This research will focus on identifying the barrier and the solutions in providing mangosteen exportation from Indonesia to global market. This is because mangosteen has comparative advantages in export markets [5]. The spread of mangosteen production centers in Indonesia is not evenly distributed, as its development is still concentrated in Java Island and Sumatra. The highest production of mangosteen is in Java Island (60,6%), while Sumatera and other islands are 24% and 15,4%, respectively [6]. West Java is highest production of mangosteen (around 49.74 ton per year), West Sumatera (around 16.50 ton per year) and Central Java (around 12.274 ton per year). During that time, the total production of mangosteen in Indonesia was 162 ton per year [7]. Therefore, this research become important to analyse the barrier factors exporting mangosteen to global market. Therefore, the information will be obtained and formulated into policy recommendation.

Methodology

The research will be conduct by systematic literature review. According to [8], a systematic review is a research method that is undertaken to review research literature, using systematic and severe methods. A systematic literature review is a means of identifying, evaluating and interpreting all available research relevant to a particular research question, or topic area, or phenomenon of interest [9]. Export barriers can be defined as various obstacles that hamper a firm’s effort to initiate, sustain or develop export activities [10]. Export barrier including any factor both internal and external that prevent companies from initiating, expending, or continuing export activities [11]. Internal barriers are those occurring within organizations, and normally associated with company resources or export marketing strategy, whereas external barriers come from outside the company, either in foreign markets or in national contexts [12]. To overcome the export barriers effectively, accurate identification of the barriers as well as their level of intensity and severity are required [13]. The exporters can anticipate or reduce their impact on export activities with a good understanding of export barriers, especially for the barriers that are within the firm’s ability to cope with. A good understanding of export barriers also helps the government agencies to provide appropriate policy measures and assistance to individual firms or business/industrial associations in their export-related activities [14].

Based on literatures, this research essay will use four commonly researched export barrier constructs, namely exogenous barriers, procedure barriers, resource barriers and knowledge barriers [13, 10, 15, 12 and 11]. This research essay will explore about the barriers related knowledge, resource, procedure and exogenous in providing mangosteen commodities exportation from Indonesia to global markets.

Results

Smallholder farmers of mangosteen in Indonesia

According to [16], smallholder farmers are family farmers including those who are small-scale producers and processors, pastoralists, artisans, fishers, community closely dependent on farms, indigenous peoples and agricultural workers. Smallholder farmers in Indonesia defined smallholders as those with a low asset base, operating less than two hectares of cropland [17].

Small farms have been defined as those depending on household members for most of the labour [18].

Barrier factors to export Mangosteen to Global Market

The growing potential of the mangosteen market is both an opportunity and a challenge for Indonesian mangosteen producers. The potential market becomes an opportunity when followed by increased production and quality of the fruits. However, it would be a threat if the Indonesian mangosteen production is not able to compete in the global market. The chance for Indonesia as the producer of mangosteen to compete in the global market is quite big. As Indonesia is supported by natural resources that are suitable for the cultivation of mangosteen in various regions of the archipelago. Various agro-climates in different regions make it possible to produce mangosteen throughout the year. The availability of the land and the diversity of its germplasm is potential to produce good quality mangosteen and for the industrial company to process raw products of mangosteen. Human resources that familiar with mangosteen cultivation are relatively large, even though their understanding and mastery of technology are still low [5]. Even though Mangosteen is very prospective and has the potential strategic role in the Indonesian economy, but the problem is very complex. The prominent problems are the production, quality, and low continuity, susceptible to quality degradation. This problem has the implications on low competitiveness of Mangosteen in global markets. There are also many export barriers that influenced mangosteen exportation from Indonesia to the global market [19].

Barrier factors for Smallholder farmers of mangosteen in Indonesia

The smallholder farmers in Indonesia as well as in other developing economies in the Asia region, face the challenges and opportunities of a rapidly changing market environment brought by trade liberalization and globalization. Rising incomes, population growth, urbanization, change in tastes and preferences are driving growth in demand for high quality and safe food [1]. During this time, mangosteen by smallholder farmers in Indonesia is not yet used as the main plant, but only as a side plant and the inheritance of the ancestors, plants in yard, so that the handling of mangosteen is still traditional [5]. There are barriers for smallholder farmers of mangosteen in Indonesia.

Knowledge Barrier

Mangosteen export from Indonesia has some problems in quality, quantity and continuity. The quality problem in marketing of mangosteen is the low percentage of eligible export fruits. From the total of Indonesian mangosteen production is less than 25% of fruits that fulfill export standard criteria. These conditions are caused by the lack of information and knowledge on the factors affected fruits quality and quantity. The existence of scars on fruit skin and fruit damaged by yellow gummy, commonly called as gamboge disorder (GD) are the main problems in decreasing fruit quality. Losses caused by scars and GD are very seriously, since the fruit with scars is unattractive and with GD is unfit for consumption and processing [20]. Scars on mangosteen fruits caused by trips are the most prominent constraint in Indonesian export of mangosteen. Most of the exported mangosteen are rejected due to scar appearance [21].

The Indonesian mangosteen production is lower than its production compares to other countries' mangosteen production. For example, Indonesian mangosteen's trees can produce about 60 kg to 100 kg per tree, while in other countries their mangosteen production reaches 200 kg to 300 kg per tree. The low quantity and continuity caused by low of productivity of mangosteen plantations. The cultivation of mangosteen is still using conventional cultivation, and the quality not qualified in importer countries [19]. This low of production is mainly due to the lack of the application on GAP by farmers [22], lack of pre-harvest and post-harvest (Good handling product (GHP)) as well as lack of quality assurance management on the farmer's level. The domestic fruit quality is affected by poor handling during harvesting and post-harvesting. The fruits have to be handpicked and separated to ensure that the pulps are free of physiological disorder [23]. The technologies to overcome these problems have been found and needs to be socialized to farmers.

The implementation of technology should be in accordance with the needs and specific location because of mangosteen grown in various agro-climate conditions. The technology is technically easy to implement, inexpensive, efficient, and adaptable [24].

Resource Barrier

Research by [25], another problem of the mangosteen smallholder farmers is lack of capital.

The mangosteen cultivated is generally a legacy plant in a relatively small-scale, less than one-hectare plantation and the management of the crop is also traditional [19]. The average land ownership for mangosteen was about 0.5-0.75 ha [24]. The limitation of capital is a classical constraint that mostly experienced by small-scale fruit farmers, especially those who only control the land of less than one hectare. Fruit cultivation requires a relatively long period of time, the first harvest needs time 3-6 years. The smallholder farmer of mangosteen also lacks production input, especially fertilizers and pesticides to support management mangosteen. In regards to human resources, mangosteen farming's are mostly done by farmers who cultivate crops and other commodities such as rice crops. This means mastery of specific management technology on mangosteen cultivation is very low. Such conditions preclude the spirit and motivation of mangosteen farmers to develop. The number of extension workers who master the technology of fruit crop management, especially the mangosteen fruit, is very few. These extension workers are mostly not skilled in mangosteen crops [19]. Indonesia's mangosteen that can be exported is less than 10% of total production and the production kept decreasing every year. This is because of the age of mangosteen tree that the average age of the mangosteen tree is old. The amount of tree per farmer is 5-225 with an average of 60 trees. However, not all trees could produce fresh and ripe mangosteen fruits. As the average number of mangosteen trees that can produce fruit are 37.3 trees, therefore the ratio of the number of trees bearing fruit with the overall amount of the mangosteen tree about 62% [26]. It should be noted that, the average age of mangosteen plants is about 10 years, thus in general, a new mangosteen plant begins its production on the year 6^{th} [24].

Procedure Barrier

Mangosteen areas are mostly scattered in community forest areas while moorlands are far from the settlement. The road facilities to the mangosteen plantation area are very limited or have not existed at all. Thus, transportation access to the area is very limited. This condition makes the management of mangosteen by farmers is not optimal and only limited to mangosteen harvest time [25]. Transportation for mangosteen from the region to the collecting warehouse takes a long time, and the risk of damage to the fruits is very high. Overall, those situations can impact to low profit that farmers received [5]. In addition to all those complicated situations are the sanitary and phytosanitary certificates that are required by the importing countries [23]. For example, when Indonesia will export the mangosteen to Australia, should fulfil the regulation include pest risk management evaluates and the establishment of quarantine pests where they have been assessed to have an unrestricted risk.

Exogenous Barrier

Climate including actual changes in weather such as temperature and rainfall increases/decreases has direct influences on agricultural production [27]. Nowadays, climate change is inevitable. Agricultural sector will face distressing effects in shape of annual damage to the production levels due to a higher temperature and water availability [28]. The climate change including extreme weather, very hot and too much raining lead to reducing the production of mangosteen [29]. Seasonality is one production cycle (the period of time that passes between one production event and the next) and occurs over twelve-month period [30]. Seasonality is not only linked to months and seasons, but also changes in supply and demand [31]. Surplus and low prices of mangosteen are problems during the main season. Research and development in staggering the production as well as processing the mangosteen into other products, such as jams and juices, could minimize this adverse effect [23]. Moreover, the sense of belonging to the mangosteen commodity among Indonesian society is still not well developed [25]. The other exogenous barrier, the sense of belonging to the mangosteen commodity among Indonesian society is still not well developed. In general, the mangosteen is only a legacy from their ancestors. That is make low sense of belonging in the new generation [19].

The solutions of the barriers for Smallholder farmers of mangosteen in Indonesia

Firstly, *improving the adaption of technology innovation.* Mangosteen production is currently very low in quality. It is because the cultivation of mangosteen is still using conventional cultivation and the quality of mangosteen is not qualified for export [32]. Improving the adaption of technology innovation including GAP, management pest and disease, GHP and pre-post-harvest by extension and capacity building will improve knowledge of farming, particularly how to produce healthy fruits [5]. Improving skills in GAP is believed to be an important tool towards revitalizing the lost power in farming [33], and also solved issues regarding to sanitary and phytosanitary requirements for exportation [23].

Secondly, *improving infrastructure*. Infrastructure is services derived from the set of public work traditionally supported by the public sector to enhance private sector production and to allow for household consumption [34]. Related to mangosteen smallholder the infrastructure that should be improved were roads, bridges, irrigation and electricity. Improving infrastructure will significantly improve economic and social conditions [35].

Thirdly, *expanding the mangosteen area and supporting agriculture production*. To improve production of mangosteen, it can be done by expanding new area that provides certified seeds and fertilizers. Research by [19] showed that the simulation of seed and fertilizer subsidy for 15% is able to increase export by 112, 13% or 16,484 ton. In addition, according to [19], the expansion of the harvest area by 50% will increase the mangosteen production by 22.36% and increase its productivity by 16.55%. Moreover, the 50% of the mangosteen harvest area will increase by 113,86% or 16,618,5 ton for export.

Fourth, *increasing sense of belonging to mangosteen*. In Indonesia the mangosteen is a national asset that must be maintained and should be the pride of every individual. The sense of belonging to the mangosteen fruit can be socialized through family education, schools, social organizations (religious, traditional and cultural), and government agencies. It can be realized in the form of laws, presidential instructions, and regional regulations, as well as regulations or customary law [25].

Fifth, *strengthening smallholder farmer's institutions by vertical integration*. Strengthening smallholder farmer's institutions including farming, dissemination, capitalization, and marketing institutions [25]. Vertical integration of mangosteen business was necessary in order to take affirmative action on the risk mitigation. Vertical integration would lessen the risk of cost increasing, disruption of critical material supplies, and quality problems [29].

Sixth, *providing leasing for mangosteen cultivation.* Nowadays, the provision of agricultural loans services is constrained by some factors. This includes being ignored by the commercial banks due to the perceived risk in agricultural financing and the negative consequences of agricultural market [19].

Conclusion

Indonesian mangosteen farmers have several limitations in mangosteen exports. Limited knowledge in improving quality and quantity is a barrier in knowledge. Small scale of land ownership, capital and limited human resources engaged in fruit agribusiness are resources barriers. The scattered production centers among the islands, limited transportation and packing house infrastructure are the barriers in terms of procedures. The problem of continuity, seasonally fruit, climate change and low prices are the exogenous barriers. At present Indonesian mangosteen farmers have more advanced through government intervention in overcoming these barriers.

Among them are the increasing production mangosteen harvest areas, through distribution of planting materials for farmers. Quality improvement is carried out through assisting farmers for cultivation, control of pest and disease, harvesting and post harvest handling and increasing the number of registered orhards which implementing GAP. Other efforts that are being and will continue to be carried out are improving road infrastructure, building packing houses, and linking farmers to the markets and industries. In addition, collaboration between farmers, local government and private sectors is important thing to accelerate mangosteen development.

Recommendation

Based on the literature analysis, there are several recommendations to overcome the export barriers. Smallholder farmers of mangosteen should develop by government intervention include:

- Improving their implementation on GAP, pre and post-harvest to increase the production and quality of mangosteen by capacity building from Ministry of Agriculture.
- The government through The Ministry of Agriculture and The Ministry of Agrarian Affairs and Spatial Planning/National Land Agency should provide the expansion of mangosteen harvest area.
- Supporting finance and technology, as well as producing logistics that significantly reduces risks for smallholders.
- Increasing farmers knowledge through dissemination and socialization of cultivation, pre harvest, post harvest and pest and deseases technology.
- Building road infrastructure and packing houses.
- Linking farmers to the market and industries.
- Providing seedlings to support development area of mangosteen production.

- Facilitating collaboration between farmers, local government and private sectors to accelerate mangosteen development.
- Increasing their sense of belonging on mangosteen by extension.

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Evaluation of Horticulture Seed Distribution Program

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Abstract

The objective of this research was to evaluate the extent to which the seed distributed on SPEKTA HORTI 2018 has been planted by farmers. Evaluation study was conducted on October-December 2018 in Lembang, Cianjur, Pemalang, and Tegal. Primary data were collected using a standard structured questionnaire. Evaluation methode used CIPP model. The results showed that dissemination of superior horticulture seeds has become a fast media for seed distribution, and provide real support for the development of horticultural areas. The seeds were received in good condition (citrus), bad (garlic), and damaged/dead (durian, potato). The damage to the seeds was due to the transportation that was far away, the rainy season and less postharvest handling. It is recommended that horticultural seed transportation get special treatment and the recipient farmers prioritized for farmers whose main job is as farmers. Technical guidance and assisting the seeds distributed is very important, to find out whether the seeds are planted, treated according to the technology recommendations and in time to produce.

Keywords: dissemination, seed, evaluation, CIPP

Introduction

The government strive to reduce poverty in Indonesia. [1] The poverty rate in Indonesia in September 2018 at the level of 9.66%. [2] A strategy that can be done to overcome poverty includes increasing the basic capacity of the poor to increase income, empowerment the poor in the overall process of poverty reduction. The research results [3], [4] concluded that community empowerment is an effective step in poverty reduction. One of the priority programs for poverty alleviation carried out by the Ministry of Agriculture is the Horticulture Seed Program by launched 2018 as the year for horticulture seeding. The program's goal was to provide certified seed massively, which contribute on self-sufficiently and food security. The effort to increase productivity should be started with quality seed supplies [5] .

Quality seeds play a role in determining crop production. Seed quality is reflected from physical, genetic and physiological quality [6], [7] and [8] states that the use of disease-free citrus seeds is important requirements to produce high-yielding citrus, especially by using certified seeds. Low garlic productivity can be caused by seed quality [9]. Most potato farmers in Indonesia do not adopt certified potato seed because of relatively expensive price of certified seed [10]. High risk of potato seed production discourages the certified seed producers to produce it in sufficient amount. The government needs to empower the farmers to produce quality seed by themselves.

Some obstacles on national seeding are related with seed genetic characteristic diversity, availability, and amount of qualified seed [11].

Indonesian Centre for Horticulture Research and Development (ICHORD) has distributed horticulture seed massively and freely to farmers on SPEKTA HORTI 2018 on September 2018.

The activity's goal was to motivate farmers to use certified seed and to accelerate technologies dissemination. Research on the effectiveness of government programs has been carried out, including research on the effectiveness of extension work by [12] and [13] which showed that the performance of extension agents has been effective in empowering farmers. But research on the effectiveness of seed distribution programs is still rare. [14] has conducted research on the evaluation of programs to provide seed subsidies in increasing rice productivity. The results of her research recommend the need for further research on a program to provide seed subsidies with a wider range. Therefore, this research is important to be carried out. The objective of this research was to evaluate the extent to which the seed distributed on SPEKTA HORTI 2018 has been planted by farmers. This lesson learned guide how to implement seed distribution program.

Methodology

Evaluation on seed distribution by ICHORD was done on December 2018. Survey was done thru interview and distribute questionnaire which should be answered by seed recipient on citrus in Lembang (22 respondents), potato in Cianjur (25 respondents), garlic in Tegal (16 respondents), durian in Pemalang and Temanggung (14 respondents). Questionnaire composed of general information of respondents, types of crops normally grown by the respondents, respondent's willingness to plant the seed distributed and to adopt technology. Evaluation used CIPP (context, input, process, product) model. CIPP approach is the following programme evaluation which is the systematic collection of information about the activities, characteristics, and outcome of programmes for use by specific people to reduce uncertainties, improve effectiveness, and make decisions with regard to what those programmes are doing and affecting patton (1986) [15]. The CIPP model of evaluation is showed in the table 1.

Table 1. The CIPP Model

Aspect of evaluation	Type of decision	Kind of question answered
Context evaluation	Planning decisions	What should we do?
Input evaluation	Structuring decisions	How should we do it?
Process evaluation	Implementing decisions	Are we doing it as planned? And if not, why?
Product evaluation	Recycling decisions	Did it work

Sources: [15]

Indicator variable on product evaluation is seed dissemination which is measured by willingness of farmers to adopt technology by measuring percentage number of farmers who want to adopt technology. Then, analyze quantitatively was done to measure some factors which affects adoption level which were formulated into the relationship of logistics functions [16]:

P=Farmer opportunity to adopt technologies (pi = 1, adopt; pi = 0, do not adopt)

1-p1=Farmer opportunity to not adopt technology

X1=Age (years)

X2=Education (years)

X3=Experiences (years)

X4=Income from farming (rp)

D1=Dummy main activities (d=1, farmer; d=0, non-farmer)

D2=Dummy commodities (d=1, same with distributed seed; d=0, different with distributed seed)

D3=Dummy gender (d = 1, male; d = 0; female)
A=Constanta or intersep; βi = regression coefficient-i (i = 1, 2, 3, ... 11)
E=Error term

Result and Discussion

Context evaluation

The Ministry of Agriculture launched that 2018 was the year for horticulture seeding. This was motivated by increasing demand and import reducing of horticulture products, as well as poverty reduction. For this reason, it is needed to increase horticulture productivity and competitiveness on strategic commodities started with qualified seed supplies. Around the world, researchers, policy makers, and foundations are working hard to improve seed provisioning to farmers in developing countries in order to increase agricultural productivity [17]. Quality seed of any preferred varieties is the basis of improved agricultural productivity [18]. ICHORD implemented this program thru seed producing massively to be distributed to farmers freely since 2017, including shallots, garlic, potatoes, durian, mangosteen, mango, “pete jengkol” and citrus. The seed distribution was started on August 17, 2018 by launched 1.000.000 citrus seed/stem.

Furthermore, horticulture seed was distributed on SPEKTA HORTI 2018 on 20 September 2018. Horticulture seeds must be distributed quickly and massively in order to develop horticulture cluster.

This program encourage farmers to produce crop from certified seed and push ICHORD to have responsibility to produce and distribute certified seed massively. This is in line with the results of research [19] which concluded that the supply of certified potato seeds was a priority strategy in increasing potato competitiveness in Banjarnegara Regency. The availability of certified potato seeds is the key to the successful introduction of certified seeds [20].

Input Evaluation

Stakeholder

There were some stakeholders who give contribution on the program showed on Table 2. Stakeholders plays an important roles to determine the system requirements. Stakeholders are related to each other and interact with each other. Interactions between stakeholders including: exchanging information, exchanging products, instructions, and providing supporting tasks [21].

Table 2. Stakeholder and its Contribution

No	Institution	Contribution
1.	ICHORD and its research institutes, such as IVEGRI, ITFRI, ICSTFRI	- Produce certified seed - Provide technologies - Provide researcher to give technical assistant
2.	Directorate General for Horticulture	- Provide data on farmer who would receive seed - Organize agriculture Services to coordinate farmers
3.	Agriculture Services	Assist farmers start from received of certified seed, planted and should be until harvesting
4.	Breeder Seed	IVEGRI, ITFRI, ICSTFRI collaborated with breeder seeds to produce seed massively
5.	Extension agent	Assist farmers start from receiving certified seed, planting until harvesting
6.	Group of farmers	Organize farmers

Source: Primary data, 2018

Providing Seed

ICHORD has distributed horticulture seed to farmers, which is showed at table 3.

Table 3. Horticulture seed which was distributed to farmers on SPEKTA HORTI 2018

No	Commodities	Amount	Area of distribution
1	Shallot: Bima variety	24 tons	16 group of farmers: Sukabumi (12 tons) Subang (4 tons), Indramayu (4 tons), Majalengka (4 tons).
2	Garlic	25 tons	Agriculture service: Tegal district (10 tons), West Java (5 tons), NTB (5 tons), Maluku (5 tons)
3	Potato	31 tons	10 group of farmers: West Bandung (15,5 tons), Purbalingga (6 tons), Banjarnegara (4 tons), Sumedang (0,5 tons), Cianjur (5 tons)
4	Durian	6000 stem	Local government Lamandau Kalteng (2000 stems), Pemalang (2000 stems), Temanggung (2000 stems)
5	Mangosteen	5000 stem	District of Solok
6	Mango	2500 stem	District of Oku Timur, South Sumatra. Cirebon
7	Pete, jengkol	3200 stem	District of Temanggung, Blitar district
8	Citrus	8000 stem	12 Group of Farmers in Bandung Barat, Pacitan, Bogor, Wonosobo

Source: Primary Data, 2018

Technical assistant

Technical assistant on cultivation of garlic, potatoes, citrus and durian was provided in order to improve farmer capability to plant seeds based on recomended technologies. [22] and [23] stated that improvement of human resources capabilities thru technical assistant regularly is important factor on community empowerment process. During SPEKTA HORTI 2018, ICHORD provide technical assistant to seed recipients and to all SPEKTA HORTI visitor. Other technical assistant was done on farmers' location.

Dissemination Method

The dissemination method used were launched distribution through "SPEKTA 2018" and publications through various media. The launching was carried out by Minister of Agriculture, who gave a strong encouragement to farmers to follow up with planting. The other dissemination method was publication through various media, so that IAARD will continue to receive attention from public as a place to obtain quality seeds and horticulture technology. In addition, IVEGRI as a place for organizing activities has become more well-known. The figure of the Minister of Agriculture could be an attraction for the media to come to cover the event. This launching event was considered successful with presence number of mass media and the amount of news coming down from online media, electronic media and print media. The mass media covering the event included 32 online media, 17 print media and 6 electronic media (five televisions and one radio).

Process Evaluation

Table 4. Evaluation of seed condition and farmers response until December 2018

Observation variable	Evaluation results			
	Citrus	**Garlic**	**Potato**	**Durian**
Seed condition	Good	Only 28% seed can be planted (over dormant period)	Damaged	Damaged due to transportation process.
Planted location	Planted on farmers' land and agro tourism	Farmers land	Farmers yard	Farmer yard
Seed Growth	Good	Some seed growth well, some seed did not growth well	Farmers have not planted distributed seed	Farmers have not planted yet (50% damaged)
Level of farmers knowledge about citrus cultivation	Only some farmers know how to cultivate citrus	Farmers have experiences on garlic cultivation	Farmers know how to cultivate potato well	Farmers have experiences on durian cultivation
Percent of respondents who already plant	95% of respondents	75% of respondents	83,33 % of respondents	100% of respondents
Farmers's reason to plant citrus	Farmers believe that IAARD seed has good quality (33%), do not need to buy seed (28%), and interested on citrus which were displayed on Spekta Horti (23%).	Farmers believe that IAARD seed has good quality (43,75%) and do not need to buy seed (25%)	Farmers believe that IAARD seed has good quality (66%) and do not need to buy seed (23%)	Farmers believe that IAARD seed has good quality (100%),

Source: Primary data, 2018

Technical assistant was done after one month of seed distribution to 30 person (citrus), 16 person (garlic), 16 person (durian), and 25 person (potatoes). Technical assistant of citrus cultivation consists of growth standard to cultivate, land preparation, citrus varieties selection.

Garlic technical assistant consists of agroecology, pH and water standard, how to qualified seed, certified seed, pest and disease protection. Durian technical assistant consist of seeding, durian cultivation, watering. Potatoes technical assist consist of potatoes cultivation, pest and disease control, fertilizer, watering, harvesting.

Product Evaluation

The expected impact of seed distribution activities is the adoption of IAARD technology innovation. The outcome of this activity is the increasing interest of farmers to adopt IAARD technology innovation. The results showed that 83 percent of farmers were interested in adopting IAARD technology because the technology was easy and believed that the seeds distributed by IAARD had good production potential. While 17 percent were hesitant to adopt because they were dependent on farmer groups. The adoption of this technology is thought to be influenced by age, education, farming experience, main work, cultivated commodities, and farming income. [24]

Understanding the factors that influence adoption of agricultural technology is essential in planning and executing technology.

The simultaneous test results on the model showed that with a confidence level of 90 percent, the probability of a statistical LR is 0.0723 so that h0 is rejected. This can be seen from the LR chi value (2) of 411.57 with probabilities of chi2 of 0.0723. In other words, the six variables simultaneously influence the adoption of technology. The goodness of fit test is carried out through the person or hosmer lemeshow test. The test results showed the value of prob> chi2 is 0.5598.

The value of prob> chi2 which is greater than 0.1 indicates the ability of the model to predict the observation value or the model is acceptable because it matches the observational data. The next step is to test each variable. The results showed that there were 2 variables that significantly affected the interest of farmers to adopt technology, namely the main work and farming income.

This is indicated by the value of prob> chi2 of the two variables which is <0.1. Table 6 showed the results of partial significance tests on factors that influence technology adoption.

Table 5. Results of partial significance tests on factors that influence technology adoption

No	Variable	Prob > chi2
1	Age	0,71
2	Education	0,91
3	Experience	0,66
4	Main activities	0,007
5	Commodity	0,20
6	Farming Income	0,10

The test results of coefficients and odd ratio values of the two variables that significantly influenced technology adoption showed that these two variables have a positive effect on adoption rates. The main occupational variable has an odd ratio of 9.17 meaning that the recipient of the seed whose main activities is farmers have 9 times greater chance to adopt technology. The income variable has an odd ratio of 1 meaning that if the income from farming increases by 1 rupiah, the opportunity to adopt technology increases with the same ratio. In line with the results of this study, [25]. The level of income is one of the factors that influence farmers' perceptions of adopter on the characteristics of innovation.

Conclusion

Dissemination of superior horticulture seeds has become a fast media for seed distribution so that the seeds reach farmers to be planted, and provide real support for the development of horticultural areas. The seeds are received in good condition (citrus), bad (garlic), and damaged/dead (durian, potato). The damage to the seeds is due to the transportation that is far away (durian) and the quality of the seeds was not good because of the results of the rainy season production and less optimal postharvest handling (garlic and potatoes). This evaluation need to continue with performance of seed has been planted.

For the success of dissemination, it is recommended that horticultural seed transportation get special treatment because of sensitivity to changes in environmental temperature. It is also recommended that the determination of the recipient farmers be more targeted, which is prioritized for farmers whose main job is as farmers. Technical guidance is very much needed by farmer groups that receive seeds, extension agents and related agencies, because it is a commodity that has just been developed (citrus in West Bandung regency which was originally the main crop are vegetables), garlic in Tegal Regency and durian in Pemalang Regency (commodities developed again after a long vacuum), and potatoes in Cianjur district (some sub-districts are new developments). Assisting the seeds distributed is very important, to find out whether the seeds are planted, treated according to the technology recommendations and in time to produce.

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The Influence of Chili Supply on Daily Prices at Kramat Jati Central Market

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Abstract

In Indonesia chili is classified as strategic commodity and has high economic value. The price of chili was allegedly strongly influenced by the supply side which is influenced by production and distribution. Kramat Jati central Market in Jakarta is categorized as regional market and functioning as national price barometer, thus the chili prices in Kramat Jati central market contribute to determine the national chili price. The purpose of this research was to provide an overview of what factors affected the price of chili in Kramat Jati central market. The data used were secondary data derived from Kramat Jati central Market, which is the daily price and supply of chili during 2012-2016. The linear regression results showed that the same day supply significantly affect the price of that day, with the R square value 29.9%, and the rest are influenced by other factors outside the model. Further calculations with the addition of variables as follows, the supply and price on the previous day. The result showed that same day supply together with the supply and price of the previous day, has a significant influence to chili price with R square 98.77%. This condition is related to the consumption behaviour of chili which is in fresh form, and due to the nature of chili which is easily damaged (perishable) so it must be sold quickly in the next day.

Keywords: chili, price, supply, regression

Introduction

Chili is a strategic commodity which classified as staple food. Through the Presidential Regulation (Perpres) Number 71 of year 2015, chili was grouped into one of the primary and important goods. This is due to its characteristics that cannot be substituted but needed every day.

Chili place an important position in the Indonesian food menu and considered as an appetite enhancer, although it is needed in small quantities, but almost every day consumed as a spice or chili sauce. Chili consumption by households in the fresh form reaches 80%, and the remaining 20% are for processing industries (Saptana *et al*., 2010; Nauly 2016), and with the development of the tourism sector which is certainly supported by the development of the culinary business, chili needs are expected to be even higher.

The price of chili is allegedly determined by supply side, which is influenced by production and distribution. Chili production is seasonal, where there is a scarcity of production in certain months, while the amount of consumption tends to be remained, furthermore on holidays or religious holidays the demand for chili increases by around 10-20% (Sativa 2017; Saptana 2012; Maramis 2015; Anwarudin *et al*., 2015). Production factors are not the main factor that causing price fluctuations, distribution factors are considered more influential. Disruption of distribution potentially caused supply scarcity which can trigger price increases, besides that the distribution chain and increase in distribution costs are also influential (Saptana, 2012). The chili price is elastic to its supply, which means when the supply is less than demand, the price will be increased, whereas when the supply is greater than the demand, the price will drop. This condition causes chili price to fluctuate, furthermore the increase in chili prices can be a cause of inflation. The market structure that tends to be oligopsonistic also affects price fluctuations, especially on religious holidays (Nauly 2016). Thus, as one of the basic necessities, the government have to ensure the chili supply is sufficient all the time so that price fluctuations can be minimized. This is because fluctuating prices can have a negative impact on farmers, traders and also consumers.

Chili that distributed from the production center areas will be marketed to regional consumption centers. Most of chili consumption centers are in big cities, which one of them is DKI Jakarta. Chili supply that meet DKI Jakarta market area and surrounding areas, such as Bogor, Depok, Tangerang, and Bekasi (Jabodetabek) will usually supply through the Kramat Jati Central Market.

In the distribution of goods, especially for vegetable and fruit commodities in the Jabodetabek area, Kramat Jati Central Market is a wholesaler market and has a very important role, because it is the only market for vegetables and fruits with a regional service scale (Yuliarti and Fitrani, 2011), and with this role, it becomes a price barometer for other markets.

The price of traders in Kramat Jati Central Market is one of the references in determining chili prices for wholesalers in various local markets in the region. Price information is generally obtained in stages, especially from wholesalers to inter-regional traders/collector traders in the countryside, then to farmers, where the main source is wholesalers. Based on the results of the chili price cointegration research, showed that wholesale chili prices in Kramat Jati Central Market affect chili prices at the producer level. This is in line with the fact that the prices prevailing at the farm level are based on prices in the wholesale market, and retailers set the selling price of chilli based on the purchase price of the wholesale market. It explains that price transmission between chilli marketing institutions runs symmetrically and prices at wholesale level have a dominant influence on price formation at the producer and consumer level (Elvina *et al*., 2017), this showed the important role of the Kramat Jati wholesale market as a barometer for regional and national prices. The price of chili in the Kramat Jati Central Market is often used as a reference by the government in monitoring price movements. Therefore, the stability of chili supply to Kramat Jati Central Market has an important role in maintaining price stability, which in turn will impact on chili prices on a regional and even national scale.

Previously many policy makers only concider about the basic rules of economy that when the supply is rising up then the price will be decreased vice versa. How much influence from supply to price needs to be known so that price stabilization policies can be taken if there is a disruption in supply. The purpose of this research is to find out the factors that influence the price of chili in the Kramat Jati Central Market. The hypothesis of this study is that supply will significantly affect the price of chili in the Kramat Jati Central Market.

Methodology

This research is an exploratory study that analyzes the influence of supply dynamics on prices formed in the Kramat Jati Central Market. The data used are secondary data, including chili daily prices and daily supply data. The data obtained from the Kramat Jati Central Market, over 5 (five) years period between 2012-2016. Furthermore, linear regression analysis is carried out with the dependent variable is chili prices on average each day, and the independent variable is supply of chili at Kramat Jati Central Market. Trial and error then were conducted to looking for the dependent variable that can give better result. In the final calculation the dependent variables used are the supply of chili on the same day, the price of chili a day before, and the supply of chili a day before.

Result and Discussion

It was mentioned earlier that the supply and price of chili fluctuated. In Kramat Jati Central Market which is a market that becomes a barometer for national level, price fluctuations can affect prices in other markets. Supply behaviour and chili prices at the during the 2012-2016 period can be seen in Figures 1 and 2.

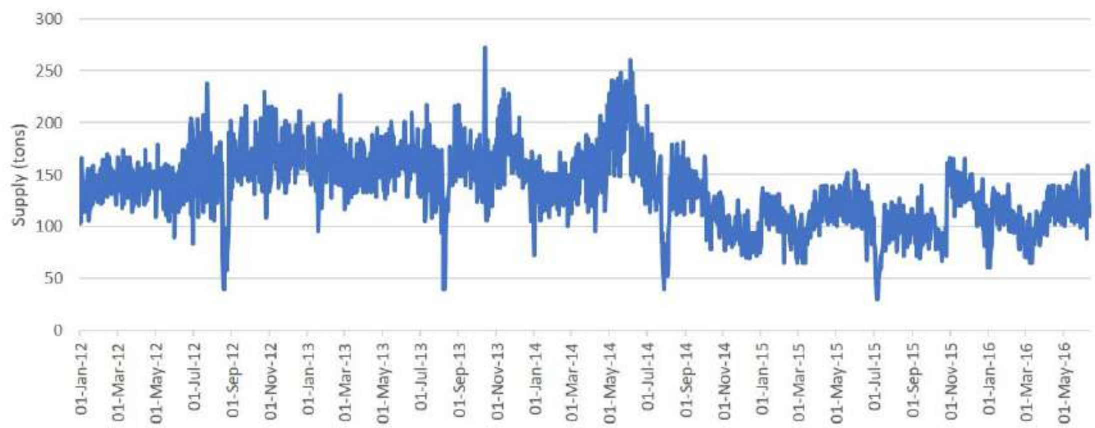

Fig. 1. Daily Fluctuation in Chili Supply at Kramat Jati Central Market in 2012-2016

Based on daily supply and price data it can be seen that the daily supply at Kramat Jati Central Market would not merely affect prices. This can be seen in figure 1 and 2 where the highest price is found in January 2015, but at that time the chili supply was not the lowest supply of chili in the period of 2012-2016. So that it can be said that the supply on the same day was not the only factor that affects prices.

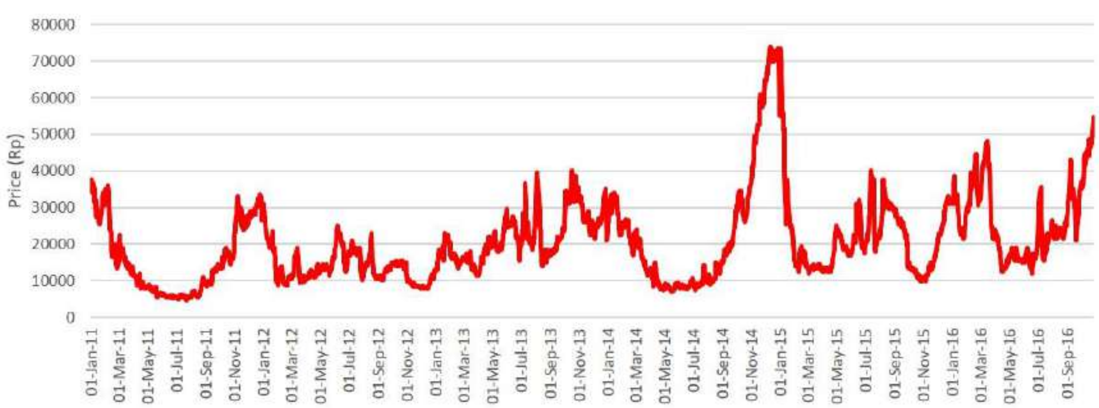

Fig. 2. Daily Fluctuation in Chili price at Kramat Jati Central Market in 2012-2016

The effect of daily supply on prices that has been analysed by regression showed that supply has a significant effect on prices with R square of 29.9%. This showed that supply on a single day will only affect the price of that day for 29.9%. While the remaining 70,1% influenced by other factors outside the model.

In this study the data used was only supply and price, therefore it was not possible to find other data outside of the two data. Although there will be a possibility that prices are affected by things other than supply. Regression analysis then continued by using available data, for the dependent variable is price, while the independent variables are the supply of the same day, the supply of a day before, and the price of the previous day.

The results of the regression analysis using price on a single day as the dependent variable and for the independent variable are supply on a single day, the number of supplies from the previous day, and the price of the previous day show the following results. The multiple linear regression equation for the pricing model is:

$$LnY = -0.026069 + 0.052895LNX - 0.040824LNX(-1) + 0.996359LNY(-1)$$

Table 1. Analysis of supply regression, supply of the previous day, and previous day of chili prices at KramatJati Central Market

Variable	Coefficient	Std. Error	t-Statistic	Prob.	
LNX	0.052895	0.018115	2.919962	0.0037	Significant
LNX (-1)	-0.040824	0.018097	-2.255859	0.0247	Significant
LNY (-1)	0.996359	0.007026	141.8089	0.0000	Significant
C	-0.026069	0.123980	-0.210265	0.8336	
R-squared	**0.987762**	Mean dependent var		9.461598	
Adjusted R-squared	0.987658	S.D. dependent var		0.631879	
S.E. of regression	0.070197	Akaike info criterion		-2.463879	
Sum squared reside	1.739453	Schwarz criterion		-2.420431	
Log likelihood	443.8025	Hannan-Quinn criter.		-2.446598	
F-statistic	9497.536	Durbin-Watson stat		1.916156	
Prob(F-statistic)	0.000000				

Method: Least Squares
Date: 11/23/16, Time: 17:38
Sample (adjusted): 2 358
Included observations: 357 after adjustments

The results of the analysis using multiple linear regression showed that the combination of supply on the same day, supply one day before, and the price of the previous day have a significant influence on the formation of chili prices with R squared of 98.77%. Based on the results of the analysis with regression can be explained several things including:

1. The effect of today's supply of LNX is significant at the 5% real level because the probability value (0.0037) <alpha 5%. The coefficient is 0.052895 which means that 1% increase in supply can increase the price of 0.052895% assuming cateris paribus.
2. The effect of day supply (t-1) LNXt-1 is significant at the 5% real level because the probability value (0.0247) <alpha 5%. Coefficient is -0.040824 means that 1% increase in supply 1 day before can reduce the price of 0.040824% assuming cateris paribus.
3. The effect of the previous price is Yt-1 significant at the 5% level because of the probability value (0.000) <alpha 5%. The coefficient is 0.996359, which mean that 1% increase in the price of the previous day will increase the current price by 0.996359% assuming cateris paribus.

The chili price a day before turned out to have a significant effect on the formation of chili prices today. This is in accordance with previous studies regarding the volatility of chili prices which stated that the movement of chili prices in that period was only influenced by the price volatility of the previous day, but was not influenced by price variants (Sumantri *et al*., 2016). The results of the analysis showed that the supply today and the supply of the previous day have a significant effect on prices. The amount supply of chili today has a direct or positive effect on prices, while the supply of one day before has a negative effect on prices. This shows that consumer willingness to pay for chili commodities is in its fresh condition, so chili with a good level of freshness will have a higher price (Reza, 2015). Pricing is not only based on demand and supply or based on cost, but also based on consumer perceived value or value of consumer perceptions of the value of the product. From the previous research said that consumers will still buy for chili even though the price is rising up (Palar *et al*., 2016; Kiloes and Puspitasari 2018).

The supply of chili on previous day will negatively affect the price of chili. If the chili supply on one day before is high it will decrease the price of chili. This is because the form of chili which is perishable so it must be sold quickly in the next day. It also showed that if the market was still available with the stock of chili from previous day, the price of the chili will decrease, and the price of chili supplied on that day is higher. That was aimed at making consumers prefer yesterday's chili supply which was cheaper, therefor the stock quickly sold out, and sellers expected profits from higher prices from the chillies that were supplied on that day. As the results of the Prastowo *et al*., (2011), traders determine the selling price based on the highest price level on the market. The response of traders when raising prices is based on consideration to maintain profits, adjust prices in the market and take advantage of moments to increase profits. In contrast, traders' response to lower prices is largely due to traders trying to reduce losses that may arise due to declining quality of goods (rotten/damaged) if they are still sold at high prices, and anticipate losing customers because they switch to other traders who sell cheaper, and adjust prices on the market.

As mentioned earlier, the price of chili is elastic to supply. The daily fluctuations in chilli supply at Kramat Jati Central Market cause price fluctuations, so the price information at the time of chili delivery does not guarantee that it will match the price when the chili arrive at the destination.

This is a risk that must be certified by chili traders. At a time when supply exceeds demand, prices will be lowered, so that the chili is sold out faster. Therefore, when compared with other strategic commodities such as shallots, the price risk for chili is higher (Sumantri *et al*., 2016).

The opposite can occur if the supply of chili is decreased then the price will be high.

Policy Recommendation

In maintaining the stability of chilli prices, the government can optimize pricing policies by controlling prices in Kramat Jati Central Market, especially through supply monitoring and arrangements. When prices decline significantly the government can implement an export policy so that abundant production will not make the price of chili drop. Conversely, when there is a scarcity of supply the government can realize an import policy to prevent higher price fluctuations.

The import export policy is considered more effective to do.

Currently the development of chili is constrained by the underdevelopment of the processing industry. On the other hand, the chili processing industry can serve as an alternative absorption of chili supply, especially when oversupply. The processing industry can also support the rapid development of the culinary industry in Indonesia which requires the consumption of chili in the form of pasta and powder. So far, most of the processed chilies in the form of pasta and powder are still supplied from imports. The production of dried red chili in 2010 was dominated by India with production more than 1000 million tons (Jagtap *et al*., 2012), followed by second place by China, and Pakistan in third place. Some of these countries supply dried chili which is a form of chili which is usually traded on world markets.

Thus, to protect farmers from losses during oversupply, and also protect traders from the risk of price drops when on the market, chili can be processed into pasta for spices or dried chili. This processed chili can be used both in the local market, for import substitution, as well as export promotion. Development of processing industries from the farm level can reduce supply fluctuations and prices that occur at the level of traders or consumers.

Conclusion

The price of chili in the Central Market of Kramat Jati Jakarta as a barometer for the price of national chili is significantly affected by the supply on that day, the supply of the previous day, and the price of the previous day, with R square value of 98.77%. It showed that the condition almost represents the actual condition. The hypothesis that supply will affect prices significantly does not only occur directly, but will also be affected by the supply and prices of the previous day.

This is related to the characteristic of chili which is easily damaged and cannot be stored for long, forcing traders to reduce prices so that chili is sold out to avoid the risk of greater losses.

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Good Agricultural Practices (GAP) in South and Southeast Asian Countries: Horticultural Farmers' Income and Capacity Building

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Abstract

In the globalization era, whereas there are no trade boundaries among Asian Countries anymore, it is necessary to enhance food safety and produce quality, environment friendly agriculture, worker's safe, health and welfare, as well as recording, traceability and reliability particularly for horticulture products. GAP is the answer of these challenges. How is the current status, implementation and outcome of the GAP implementation? This paper aimed to evaluate the implementation of GAP in Asian Countries as well as strengthening networks inter and among Asian Countries. Tabulation and descriptive analyses were employed. Results showed that among 11 South and Southeast Asian Countries, Korean, Thailand and Indonesia are the leaders of GAP implementation. However, the replication of GAP for more targeted commodities and more targeted areas should be done. The GAP implementation has been showed the outcome such as improving income of the farmers, quality of horticulture products, capacity building of the stakeholders and strengthening network of the stakeholders.

Keywords: GAP implementation, South and Southeast Asia, horticultural products

Introduction

In Southeast Asian countries, a significant development is visible in growing demand on quality and high-quality food products. On the other hand, the possibilities for export in particular of vegetables and fruits are rising very much. However, the use of chemical input increase and harm the consumers (Nguyen and Boehme, 2013). Therefore, the interest for safe and high-quality food produced under GAP have been increasing in recent years. Many developing countries are facing challenging national and international trading environments in the light of WTO, free trade agreements, multiplication of governmental GAP standards and private sector requirements. GAP is one program for safety and quality assurance particularly to protect local consumers assuring the safety of fruits and vegetables produced and traded; enhance confidence of foreign markets on safety of locally produced product; and assure the acceptance in target markets of the exporters' national programs and/or protocol (Amekawa, 2009).

Currently, GAP program has been introduced into many Asian countries for quality and safety assurance of their major crops. Technology and policy information on food safety management and GAP program which meets the agricultural environment of participating countries will be discussed by networking among experts of participating countries. A couple of model manual on GAP at primary production level of fresh fruit and vegetables that can be practically used in farm should be developed and disseminated. GAP will be optimally implemented by combining with Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP).

Water quality, manure, good handling practices, including personal hygiene and equipment sanitation, and traceability are identified as critical farm problems that, if addressed properly, are likely to decrease risk associated with microbial contamination of fresh products. However, the diverse nature of production strategies used by farmers presents multiple options for addressing food safety issues while simultaneously creating potential complications (Parker, 2012). Pesticides and fertilizers are agricultural inputs that dominantly contaminated water. Since water quality is an important component of GAPs, farmers who have already implemented this system would help reducing pollutant in the water and keeping the environment safety.

In Indonesia, it is said by Anugrah and Rivai (2011) that every agribusiness company must always adhere to the principles of GAP in order to gain market access especially on the international market as the standard of sustainable agriculture practices is one of the consumers' preference attributes of agricultural products. Furthermore, in Thailand, as said by Schreinemachers *et al*., (2012), GAP is introduced to increase the supply of safe and high-quality food by promoting a more sustainable crop production that uses fewer pesticides. In Bangladesh, urgent need to implement GAP is based on the need to reduce food toxicity to a great extent to ensure safe food (Sultana and Muhammad, 2018). In Cambodia, it is known that the implementation of GAP standards in Cambodian legislation should be speeded up (Schripsema and Broeze, 2016). Moreover, in 1990s, in order to ensure high quality and food safety, the Korean government instituted schemes such as the GAP, agricultural product origin verification, and the agro-product standardization. These schemes disclosed product information to the customers and set high standards of hygiene and grading (Park *et al*., 2019). In Lao, it is stated that the government can develop policies that will assist different actors in the value chain; for example, promotion of GAP for farmers and good manufacturing practice (GMP) especially for millers to improve rice grain quality (Fukai *et al*., 2019). Meanwhile in Nepal, it is known that accredited institutions in both government and private sectors to ensure GAP, standard procedure, and quality assurance system are scarce (Pokhrel and Pant, 2009). In Filipina, it is needed to do following strategies in order to promote GAP: intensification of awareness campaigns for the implementers; stepping-up of efforts on creating consumer awareness of GAP; focusing awareness campaigns on product attributes that consumers value; and harnessing collaborative potential of public and private sectors to effect increased market performance, long-term capacity building, and sustainability (Mojica *et al*., 2013). Additionally, Sri Lanka aims to increase national productivity of paddy up to 5.3 Mt/ha by 2025 by using adoption of environment-friendly good agricultural practices such as judicious use of agrochemicals, Integrated Plant Nutrition System, and IPM, as well as the use of mechanized farming techniques preferred by the young farmers to increase the cropping intensity and the introduction of crop diversification to increase the overall farm profitability (Wanigasundera and Attapatu, 2019). Furthermore, My *et al*., (2013) said that in Vietnam, there is VietGAP which is a national food quality standard referring not only to quality and safety aspects, but also to sustainability aspects. To promote VietGAP rice, the key is to understand consumers' knowledge and trust in food quality certification.

This paper will explain about GAP-related activities in several Asian countries and provide information, policy assistance and the capacity necessary to deal with agri-food safety issues in each country. These activities, focusing on information provision, technical assistance and capacity building, help member countries cope with food system globalization and GAP standard proliferation and are highlighted below detailed by region, activity, commodity and country.

Moreover, this paper will show the outcome of the GAP implementation, particularly to improve farmers' income in Asian countries. The aim of this study was to analyze the stage of different types of GAP introduction in South and Southeast Asian countries starting 2012 and to compare with the situation in 2017.

State of the Art of GAP Implementation in South and Southeast Asian Countries

State of the art of each country in implementing GAP is quite different. Several Asian countries, such as Indonesia, Thailand, Philippines, Vietnam, China, Malaysia Japan, Singapore have been implementing GAP since beginning, as ASEAN GAP. In Vietnam, farmers started to introduce an adopted regulation for the production of fresh fruit and vegetables under VietGAP, which was based on ASEAN GAP, Global GAP and Freshcare (Nguyen and Böhme, 2013). However, GAP for Bangladesh, Mongolia, Nepal, Sri Lanka and Bhutan is quite new. The initiation of GAP started with the idea of Codex Alimentary.

The European Countries, such as Germany, England, Spain, are the first nations implemented GAP, called EUROGAP. After several years, we know the Eurepgap as Global GAP. The GAP in European Countries is not only good for fresh food, but also good for herbal medicines. In China, the implementation of GAP for medicinal herbs started in 2002 and became regular practice, the medicine produced based on this GAP is acknowledged from the WHO (Leung PC and Cheng KF, 2008). The quality of medicine using medicinal plants produced following GAP improved significantly (Scholten, 2003). After that, Australia was developed the GAP and QA Program by combining the fresh care, Global GAP, SQF 1000 and 2000, HACCP for 4.000 supply chain business, wool worth QA for 570 supply chain business, and ISO 9002, HACCP with 50 supply chain business. Moreover, The European countries also have Good Agriculture Environmental Conditions (GAEC), that is mostly considering two pillars that are protection soils against erosion and maintenance soil organics matters and nutrients (Borrellia *et al*., 2016). Water qualities should be considered as well. Agricultural diffuse water pollution remains a notable global pressure on water quality, posing risks to aquatic ecosystems, human health and water resources. European countries develop legislation to protect water bodies (Taylor, 2016).

South-Korea introduced the GAP system and improved it started by Korean Food Research Institute and other relevant industries. They developed the act for produced Quality and Safety Management (Scitovsky, 1985). Malaysian Government under the Department of Agriculture has been implemented GAP, organic farming and integrated farming system. In line with the changes of consumer preferences, several aspects such as soil health, nutrient management, water management, pest management, energy, biodiversity need to be revised (Shobri *et al*., 2016).

Following the Global GAP, US FDA in 1998 published GAP as guide to minimize microbial Food Safety Hazard for Fresh Fruits and Vegetables. GAP in USA consider several biophysics factors such as tillage, soil management in context of sustainability (US EPA, 2015).

Thought that GAP is very important to be developed, some developing countries started already 2012 several activities supporting the development of GAP (Table 1). The stage of implementation GAP is in this year quite different. No any information related to introduction of GAP in the countries as Bangladesh, Mongolia, Nepal and Sri Lanka are available for 2012. In this time Indonesia, South-Korea and Thailand are considering advance in implementing GAP. While Cambodia, Laos and Philippines were still at the beginning. From the 7 countries they have been developed the legislation, guidelines and training at the different stages. Some of them have been impactful, but the other still in progress.

Table 1. Status of GAP Implementation in Countries of South and Southeast Asia in 2012

Country	**GAP**	**Activities**			
	Implemented	**Legislation**	**Guidelines**	**Training**	**Impact**
Bangladesh					
Cambodia	√	√		√	
Indonesia	√	√	√	√	
South-Korea	√	√	√	√	√
Laos	√	√		√	
Mongolia					
Nepal					
Philippines	√	√	√	√	
Sri Lanka					
Thailand	√	√	√	√	√
Vietnam	√	√	√	√	

Source: AFACI, 2012

A comparison was undertaken of the investigated South and Southeast Asian countries related to GAP implementation and the activities for impact, legislation, training and guidelines (Figure 1). It is obvious that in 2012 South-Korea is the advanced country in implementing GAP among the other investigated Asian countries. Even most of the countries have implemented GAP and conducted training, however in term of establishing the legislation, preparing the guidelines, South Korea is an advanced country in this regard. Besides South Korea, Thailand already has concern in the food safety from farm to table since 2005. GAP was announced as a standard for producers and be promoted ever since. There are two GAP standards in Thailand; Thai Q GAP and THAIGAP. Thai Q GAP standard is mandatory for export, and THAIGAP standard is for domestic hypermarket. THAIGAP maintained a similar structure of GLOBALGAP checklist.

Implementation of GAP in Thailand has established the legislation, guidelines, training and already has an impact (Korpraditskul and Ratanakreetakul, 2015). Mongolia, Nepal and Sri Lanka still need support from internal and other countries to implement the GAP. More information, data, lesson learned should be collected by these countries in order to strengthen the GAP implementation. In this case, collaboration related to GAP among Asian countries is needed to empower each other.

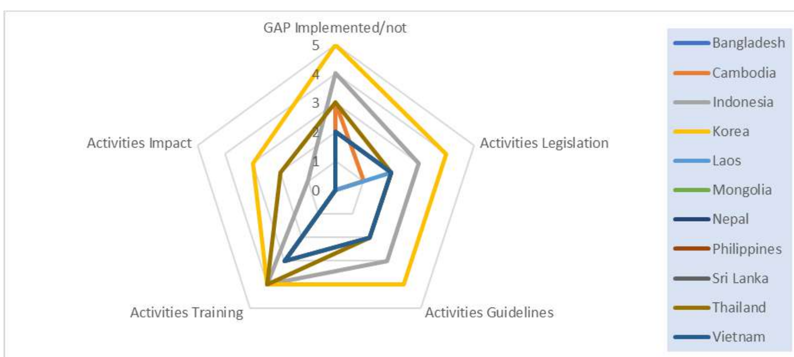

Fig. 1. Spider Web Chart of GAP Implementation Status in Countries of South and Southeast Asia in 2012 (AFACI, 2012)

GAP Implementation in South and Southeast Asian Countries

11 countries were selected in this study because they are trying to develop collaboration in implementing the GAP in frame of ASIANGAP (Layese, 2011). The advanced countries share

knowledge and information on that to the develop countries. The efficient and effectiveness evaluation of the activities have been done, so that the following countries can learn the success story from others. Some activities done, such as baseline survey, development the GAP manuals, improving the existing GAP and legislation, workshops, visit and trainings for stakeholders, establishing demonstration farm, implementing the GAP, monitoring and evaluation of the project activities, reports and feed-back.

In 2017, the status of GAP implementations in all countries have been changed. All countries except Mongolia have been implemented GAP. Regarding Mongolia the reason for missing of GAP implementation could be absent of any export of horticultural products, but nevertheless establishing of GAP will be important for the farmers and consumers, at least several trainings have been started by the extension services (Delgermaa *et al*., 2017). These countries as Indonesia, South Korea, Philippines, and Thailand implementing GAP already 2012 (Table 1) continued to improve the implementation and increased the competitiveness of their horticultural products (Table 2). Indonesia and Vietnam revised and disseminated an improved new manual. In term of dissemination to the farmers, several factors should be considered such as economics, risk, environmental outcomes, farmer networks, characteristics of the farm and the farmer, and the ease and convenience of the new practices (Kuehne *et al*., 2017).

Table 2. Status of GAP Implementation in Countries of South and Southeast Asia in 2017

Country	**GAP**	**Activities**				**Output**
	Implemented	**Legislation**	**Guidelines**	**Training**	**Impact**	
Bangladesh	√		√	√		2 GAP manuals on mango and tomato; Bangladesh GAP
Cambodia	√	√	√	√		4 GAP manuals on tomato, chilli, melon, mango; Qualified trained personnel, training materials on GAP
Indonesia	√	√	√	√	√	Revise 4 GAP manuals for chili in Ciamis, chilli in Lembang, mango in Cirebon and Salacca in Sleman with appropriate technologies; Information System on GAP; list of GAP experts; GAP network
South Korea	√	√	√	√	√	GAP manuals
Laos	√	√	√	√		GAP manuals
Mongolia				√		3 GAP manuals on tomatoes, potatoes, cucumbers
Nepal	√		√	√		GAP manuals on coffee and tea
Philippines	√	√	√	√		52 certified farmers on PhilGAP
Sri Lanka	√		√	√		GAP manuals for fruits, vegetables and leafy vegetables
Thailand	√	√	√	√	√	GAP manuals on mango and vegetables
Vietnam	√	√	√	√	√	Revised manuals

Source: AFACI, 2017

The role of government and extension agents in the implementation of GAP cannot be ignored.

In most countries, government plays a central role in guiding the evolution of research and extension system. The role of government remains central in agricultural innovation system (AIS) through sets of policies and regulatory frameworks. Therefore, the government has special responsibility to support research and extension so they can persuade farmers to implement GAP (Delgermaa, 2017).

Regarding GAP Implementation in 2017, in South and Southeast Asian Countries, South Korea, Indonesia and Thailand are the leading countries in most of the activities compare to the other countries (Figure 2). Countries such as Mongolia, Nepal, Sri Lanka have been moved forward and made progress during the 5-year collaborations in the ASIANGAP group. These three countries have learned so much from other countries how to implement GAP, but still do not give the real impacts, such as improving the techniques and system of GAP in their countries.

Hopefully in the future these three countries could develop GAP continuously and improve safety and quality of fresh fruits and vegetables. Meanwhile, in Indonesia, South Korea, Thailand, and Vietnam, GAP implementation has shown its impact whether for farmers, consumers, or even population in general. Farmers and their families obtained healthy and good quality food to assure their nutrition and nourishment, and generating a value added in their products to access markets on a better way. Consumers enjoyed better and safe quality food, with sustainable production. The population in general got benefit from a better environment (Izquierdo *et al*., 2007).

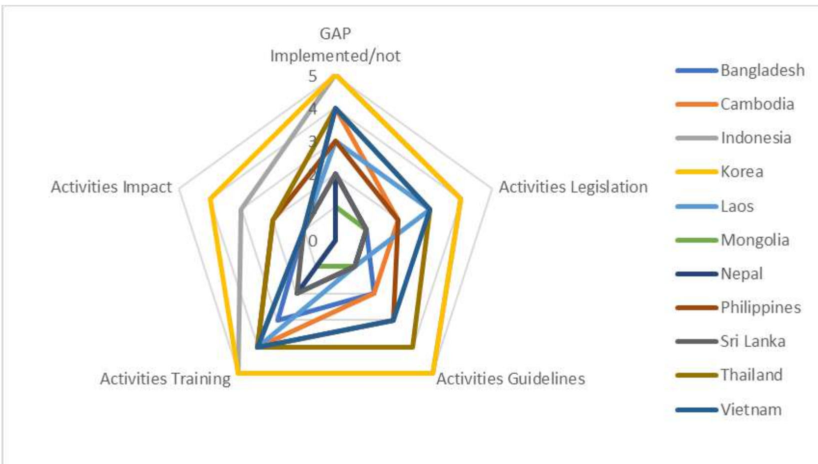

Fig. 2. Spider Web Chart of GAP Implementation in South and Southeast Asian Countries in 2017 (AFACI, 2012)

Outcomes and Impacts of GAP Implementation in South and Southeast Asian Countries

The major beneficiaries of GAP implementation would be mainly government food safety authorities, researchers, farmers, educators (professors, teachers), and private food companies, and exporters. The model manual on GAP of major fruit and vegetable crops will be practically used in other developing countries in Asia.

Table 3. Outcomes and Impact of GAP Implementation

Country	Outcomes	Impacts
Bangladesh	Training lecturers, seminars, trainings, certifications	Increase of mango exports
Cambodia	Increase of farmers' knowledge and skill	Increase of farmers' profits; reduce use of chemicals
Indonesia	Higher yield; reduce pesticides, improve awareness of farmers	Increase of farmers' income 15-35%; increase of food qualities
Korea	More farmers implemented GAP	Increase of farmers' income
Laos	Better knowledge, better standard and quality of products	Increase of farmers' capacity building
Mongolia	Increase farmers' knowledge	
Nepal	Increase competitiveness of products	
Philippines	Improve the value chain, adoption of organic/GAP	
Sri Lanka	Improve quality of products	Increase of export markets
Thailand		Increase of farmers' income
Vietnam	Increase of productivity and quality	Increase of farmers' income

Source: AFACI, 2017

Conclusions and Suggestions

As conclusions, GAP implementation in several countries is necessary to be done. It has been showed outputs, outcomes and even impacts particularly for the farmers. Several impacts are improving income of the farmers, quality of horticulture products, capacity building of the stakeholders and strengthening network of the stakeholders. In the future, more countries should implement GAP widely in order to gain more benefit in the global markets. It would be better if every country has strict regulation to support GAP like The Clean Water Act (CWA) in US. They have implemented pollution control programs and developed national water quality criteria. The CWA made it unlawful to discharge any pollutant from a point source into navigable waters, unless a permit was obtained. That way, farmers would reduce chemical compounds used in their lands and be more aware of environment (US EPA, 2015).

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COLORING THE WORLD WITH ORNAMENTAL PLANT INNOVATIONS FOR PROSPERITY

Phenotypical Characterization of Bird of Paradise (*Strelitzia Reginae* L.) Genotypes

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Abstract

Forty cultivars of Bird of Paradise genotypes were studied for genetic variability. The PCV and GCV were recorded high for number of leaves (25.37 & 26.13), number of bracts (21.40 & 28.19), number of flowers per clump (20.57 & 28.64) indicating the existence of wide range of genetic variability. The narrow difference between the GCV and PCV were recorded for all characters, indicating the lesser influence of the environment in the expression of these traits and presence of strong inherent association. High heritability estimates were observed for plant height (94.77), leaf length (72.32), number of leaves (94.29) and leaf width (65.01) indicating the predominance of additive gene component. The number of flowers per plant exhibited highly significant and positive association with plant height and number of leaves, suggesting the possibility of indirect selection of this trait. The quality traits can be improved through the selection of these characters *i.e.*, stalk length, stem girth and number of bracts. The highest inter cluster distance was observed between cluster III and IX (193.01) and the lowest between cluster VIII and X (19.70). The highest intracluster distance was observed in cluster II (18.13) and lowest in cluster VII (11.05). So, by intercrossing the divergent genotypes, *i.e.*, with maximum intercluster distance, we can utilize hidden variability and produce good recombination's in advancing generations.

Keywords: Strelitzia reginae L.*, morphological characterization, GCV, PCV*

Introduction

Bird of Paradise [*Strelitzia reginae* (L.)] an evergreen perennial herbaceous flowering plant, with chromosome number (2n=22) is grown in the regions having moderate subtropical climate.

The major producing countries of Bird of Paradise on commercial scale are USA, Israel and South Africa. The genus *Strelitzia* includes five species namely, *S. nugusta, S. reginae, S. kewensis, S. nocoli and S. candida* of them *Strelitzia reginae* is very popular flowering species growing up to a height of 90 cm, the leaf stalk is about 45 cm long with the same length of leaf blade. The flowers with orange sepals and purple petals are very brilliant, emerging from the purplish spathes on a stem about 90 cm long.

India has been identified as one of the major forces in the world floriculture scenario. With the liberalization of Indian economy, floriculture has become a new rising industry in agribusiness.

Karnataka has a prominent position on floriculture map on India. Bird of Paradise is grown commercially in India in places having tropical climate like Andhra Pradesh, Karnataka, Tamil

Nadu, Maharashtra and Kerala. In Karnataka in and around Bangalore and the entire part of transitional belt seems to be very ideal for its cultivation on account of favourable climate, soil and other factors [1].

Before taking crop improvement programmes in any crop species, a thorough knowledge on the amount of genetic variability for various characters existing in the crop is essential.

Information on nature and magnitude of variability in the existing plant material and association among the various characters is a prerequisite for improvement in the flower yield.

Material and Methods

Forty cultivars of Bird of Paradise were collected from Horticulture Research Station Kanbargi farm, Belgaum district, Karnataka were used in the studies. The RCBD design with three replications with a spacing of 60 cm x 90 cm was laid out in the research fields of Department of Biotechnology and Crop Improvement KRC College of Horticulture, Arabhavi, Gokak, Karnataka, India. The data were collected on various parameters during vegetative and flowering period from all the plants in each treatment. Observations were taken on growth viz., plant height (cm), number of leaves, number of suckers per plant, leaf length (cm), leaf width, and yield parameters viz., number of flowers per plant, flower stalk length (cm), girth of flower stalk (mm), number of bracts per flower and leaf shape at monthly intervals, up to 9 months.

Analysis of variance was carried out as per the procedure given by [2] using the mean values of random plant in each replication from all treatments to find out the significance of treatment effect.

The coefficient of variation (CV) being a standardized form of variance is useful for comparing the extent of variation between different characters with different scales [3]. Genotype and phenotypic coefficient of variation were estimated according to [4] based on estimate of genotypic and phenotypic variance. Heritability in broad sense was calculated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage [5].

Path coefficient analysis suggested by [6] and illustrated by Dewey and Lu [7] was carried out separately to know the direct and indirect effects of the important component traits on flower yield per plant. Standard path coefficients which are the standardized partial regression coefficients were obtained by solving the following set of ‘p’ simultaneous equations through the use of ‘Doo-little technique’ as given by [8].

D^2 statistics was used for assessing the genetic divergence between population comprising 40 genotypes [9]. Using all D^2 values, the genotypes were grouped into clusters using Tocher’s method as described by [10]. The intra- and inter-cluster distances were calculated by the formula given by [3].

Results and Discussion

The investigation on genetic variability, correlation, path analysis, divergence and molecular characterization was carried out using 40 genotypes of Bird of Paradise (*Strelitzia reginae* L.) during 2015 to April 2016. The results of the analysis of variance for 9 characters under study are summarised and the variance due to treatments (genotypes) was highly significant for 9 characters viz., Flower length, leaf length, leaf width, number of bracts, number of leaves, plant height, stalk length, stem girth, number of flowers per clump.

The estimates of mean, range, genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability (h2), genetic advance (GA) and genetic advance as per cent over mean (GAM) were worked out for 9 plant traits (Table 1).

The plant height ranged from 68.01 cm to 115.92 cm with a mean value of 89.57 cm. The GV (191.97) and PV (202.55) were very high. The moderate genotypic coefficient of variation (15.46) %) and phenotypic coefficient of variation (15.89%) with high heritability (94.77%) and moderate GA (27.78) and high GAM (31.02%) were noticed for this trait. Number of leaves ranged from 13.45 to 26.46 with mean value of 21.19 cm. The GV (28.92) and PV (30.67) were very high. The high genotypic coefficient of variation (25.37%) and phenotypic coefficient of variation (26.13%) with high heritability (94.29%) and moderate GA (10.75) and high GAM (50.75%) were noticed for this trait.

Leaf length ranged from 19.36 cm to 42.49 cm with a mean value of 30.02 cm. The GV (23.78) and PV (32.88) were very high. The moderate genotypic coefficient of variation (16.24%) and phenotypic coefficient of variation (19.10%) with high heritability (72.32%) and low GA (8.54) and high GAM (28.46%) were noticed for this trait. Leaf width ranged from 9.48 cm to 13.56 cm with a mean value of 11.51 cm. The GV (1.24) and PV (1.90) were very low. The low genotypic coefficient of variation (9.67%) and phenotypic coefficient of variation (11.99%) with high heritability (65.01%) and moderate GA (1.84) and high GAM (16.07%) were noticed for this trait.

Flower length ranged from 17.63 cm to 22.55 cm with a mean value of 20.64 cm. The GV (1.01) and PV (3.74) were low. The low GCV (4.87%) and moderate PCV (9.37%) were observed. Low heritability (27.09%) along with low genetic advance over mean (5.23%) and low genetic advance (1.08) were noticed for this trait. Similar results were obtained by Beura *et al*., (1995) and Mishra *et al*., (2001) for days to first flowering in dahlia and Mathad *et al*., (2003) in African marigold. Number of bracts ranged from 6.86 cm to 19.24 cm with a mean value of 13.04 cm. The GV (7.78) and PV (13.50) were very low and moderate respectively. The moderate genotypic coefficient of variation (21.40%) and phenotypic coefficient of variation (28.19%) with moderate heritability (57.64%) and low GA (4.36) and high GAM (33.47%) were noticed for this trait. Stalk length ranged from 40.11 cm to 78.39 cm with a mean value of 56.11 cm. The GV (89.83) and PV (180.70) were very high. The genotypic coefficient of variation (16.38%) and phenotypic coefficient of variation (23.24%) was very high with moderate heritability (49.71%) and moderate GA (13.76) and high GAM (23.80%) were noticed for this trait.

Stem girth ranged from 9.06 cm to 20.33 cm with a mean value of 14.29 cm. The GV (4.58) and PV (9.73) were low. The moderate genotypic coefficient of variation (14.77%) and high phenotypic coefficient of variation (21.52%) with moderate heritability (47.13%) and low GA (3.03) and high GAM (20.89%) were noticed for this trait. Number of flowers per clump ranged from 1.41 to 3.79 cm with a mean value of 2.53 cm. The GV (0.27) and PV (0.52) were very low.

The moderate genotypic coefficient of variation (20.57%) and phenotypic coefficient of variation (28.64%) with moderate heritability (51.59%) and low GA (0.77) and high GAM (30.44%) were noticed for this trait.

Genetic divergence

Forty genotypes of Bird of Paradise were assessed for 9 characters and data obtained was subjected to D^2 statistics to assess the genetic diversity. Ten clusters were constructed using Tocher's method. The relative contribution of different characters for genetic divergence (D^2) is given in Table 2. Leaf length 38.75%, Leaf width 2.95%, Number of bracts 1.54%, Stem girth 0.38%, Stalk length 0.51%, Number of leaves 31.67%, Number of flower stalk per clump 0.26%, Plant height 21.28% and Flower length 2.66%.

By following Tocher's method, 40 genotypes were grouped into ten clustering by treating estimated D^2 values as the square of the generalized distance. The distribution of entries into various clusters was given in Table 3.

Cluster I was the largest having 16 genotypes followed by cluster II (10), cluster III (6), cluster VII (2) and cluster IV, V, VI, VIII, IX, X had one genotype each. Intra-cluster and inter-cluster average D^2 values are presented in (Table 2). Among the ten clusters, cluster II with 10 genotypes showed maximum intra-cluster diversity (D^2=18.13) followed by cluster I (D^2=14.74) having 16 genotypes and cluster III (D^2=11.58) having 6 genotypes. The clusters IV, V, VI, VIII, IX and X had no intra-cluster distance (D^2=0.00) as they possessed single genotype.

Based on distances between clusters, i.e., inter-cluster distances, the maximum divergence was observed between clusters III and cluster IX (D^2=293.69) followed by clusters III and X (D^2=193.01), cluster V and IX (D^2=171.76), cluster IV and X (D^2=150.94), cluster III and VIII (D^2=136.04), cluster II and III (D^2=130.86), cluster VII and VIII (D^2=120.21), cluster VII and X (D^2=116.64) and the least is cluster I and cluster IV (D^2=20.24).

Conclusion

In Bird of Paradise, a wide variability exists among the genotypes. The plant growth and flower characters can be improved through the selection of characters like number of leaves, leaf length and plant height. The numbers of flowers per plant can be increased by the direct selection of characters like stem girth, plant height, number of leaves stalk length and number of bracts per flower.

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Table 1. Estimates of range, mean, components of variance, heritability and genetic advance for plant growth and flower parameters in Bird of Paradise

SL No.	Character	Range	Mean ± S.Em	GV	PV	GCV	PCV	h^2	GA	GAM
1	Plant height	68.01-115.92	89.57±1.88	191.97	202.55	15.46	15.89	94.77	27.78	31.02
2	Leaf length	19.36-42.49	30.02±1.74	23.78	32.88	16.24	19.10	72.32	8.54	28.46
3	No of leaves	13.45-26.46	21.19±0.76	28.92	30.67	25.37	26.13	94.29	10.75	50.75
4	Leaf width	9.84-13.56	11.51±0.47	1.24	1.90	9.67	11.99	65.01	1.849	16.07
5	Flower length	17.63-22.55	20.64±0.95	1.01	3.74	4.87	9.37	27.09	1.08	5.231
6	No of bracts	6.86-19.24	13.04±1.38	7.78	13.50	21.40	28.19	57.64	4.36	33.47
7	Stalk length	40.11-78.39	56.11±5.96	89.83	180.70	16.38	23.24	49.71	13.76	23.80
8	Stem girth	9.06-20.33	14.29±1.37	4.58	9.73	14.77	21.52	47.13	3.03	20.89
9	No of flowers/clump	1.41-3.79	2.53±0.29	0.27	0.52	20.57	28.64	51.59	0.77	30.44

GV = Genotypic variance PV = Phenotypic variance GCV = Genotypic coefficient of variance GA= Genetic advance h2 = Heritability (broad sense) PCV =Phenotypic coefficient of variance GAM = Genetic advance (per cent mean)

Table 2. Average intra and inter-cluster D^2 values of 10 clusters for 9 characters formed by 40 genotypes of bird of Paradise

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**14.74**	44.22	49.4	20.24	24.18	28.58	48.38	69.24	126.86	96.41
II		**18.13**	130.86	75.85	51.34	29.07	61.04	29.78	54.77	41.23
III			**11.58**	46.66	32.98	99.16	143.67	136.04	293.69	193.01
IV				**0**	45.96	39.74	39.51	116.45	161.51	150.94
V					**0**	56.82	98.92	39.68	171.76	75.58
VI						**0**	45.87	69.39	78.49	102.8
VII							**11.05**	120.21	69.5	116.64
VIII								**0**	99.67	19.7
IX									**0**	76.71
X										**0**

Note: Bold and diagonal values indicate intra-cluster distances

Table 3. Classification of Bird of Paradise genotypes into different clusters based on D^2 value

Cluster number	Number of genotypes	Name of the genotypes
I	16	Variety 31, Variety 30, Variety 35, Variety 20 Variety 12 Variety 15 Variety 28 Variety 7 Variety 3 Variety 13 Variety 1 Variety 21 Variety32, Variety 5, Variety34, Variety29
II	10	Variety 8, Variety 9, Variety26, Variety 10, Variety 23, Variety 6, Variety 4, Variety 22, Variety 27, Variety19,
III	6	Variety39 Variety40 Variety 38, Variety 37 Variety 36 Variety 14
IV	1	Variety 11
V	1	Variety 33
VI	1	Variety 2
VII	2	Variety 16, Variety 17
VIII	1	Variety 25
IX	1	Variety18
X	1	Variety 24

Effect of Aqua Pack with Holding Solutions on Quality and Vase Life of Gerbera (*Gerbera Jamesonii*) Cv. Dana Ellen

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Abstract

In floriculture industry, it is mandatory to maintain the standards of making bouquets and floral arrangements with modern technologies to improve the vase life. Aqua pack could be an innovative idea for increasing the vase life of hand-timed bouquets and floral arrangements to provide holding solution at the cut end. Standardizing effective holding solution for aqua pack is the present-day need in floral industry. Hence, the present lab investigation was carried out in the Department of Horticulture, Annamalai University, India, during 2017-2018 in a Completely Randomized Design with three replications. The 19 treatments comprising $AgNo_3$ @ 25 and 50 ppm, Benzyl Adenine @150 and 200 ppm and 8-HQS @ 200 and 300 ppm as individual treatments and in different combinations along with control were tested on gerbera flower stalks of Cv. Dana Ellen in aqua pack containing 200 ml of different holding solutions for each treatment. The flowers were kept in ambient condition for assessment of post-harvest parameters, quality and vase life.

The result of the experiment revealed that aqua pack with $AgNo_3$@25ppm + 8 HQS@300ppm (T_{10}) as holding solution showed greatest influences over important quality characters and vase life when compared to other treatments. There was a drastic enhancement in the vase life up to 11.95 days in the best treatment (T_{10}). The cost economics also revealed that T_{10} was observed as most cost effective (Total Cost: Vase life Ratio @3.72) holding solution to maintain quality and obtain double the vase life in aqua pack.

Keywords: Aqua Pack, Benzyl Adenine, Gerbera, Quality characters, Bouquets, Floral arrangements, Vase life

Introduction

Gerbera (*Gerbera jamesonii)* is an important cut flower grown throughout the world in a wide range of climatic conditions. It belongs to composite family. It has numerous varieties with commercial importance in the floriculture industry. The wide range of colours and attractive shape of flowers suits very well in cut flower trade across the globe. It has occupied 5th position in the world cut flower trade after rose, carnation, chrysanthemum and tulip. In recent years, gerbera is gaining commercial importance in high profile events as it is widely used for floral arrangements and flower bouquets [1]. The cut flower trade has increased many folds in international and domestic markets. The beauty of the flower lies with the freshness of the flowers for longer time without losing its aesthetic value. All along the marketing channel, there is enormous loss in the value of cut flowers which could be 50 per cent of the farm value [2]. Vase life is an important parameter for evaluation of cut flower marketability and keeping quality of flowers are affected by internal as well as external factors. The internal factors which are responsible for the keeping quality of cut blooms depend on rate of water absorption and transpiration. Respiration is another internal factor affects life of cut flowers. Some environmental factors such as temperature, relative humidity and wind velocity also affect cut flowers life [3]. On other hand, the cut flowers are deprived of their natural resources of water and nutrients after being detached from the mother plant, life process are at the expense of reserved food materials [4]. Hence, addition of chemical preservatives as supplements to the holding solution is recommended to prolong the vase-life of cut flowers in floral arrangements and bouquets, which is very essential parameter of florist industry.

All holding solutions contain two components *viz*., sugar and germicides. As sugar provides a respiratory substrate, while the germicides control harmful bacteria and prevent plugging of the conducting tissue [5]. In the same aspect, Acharyya *et al*., [6] revealed that treatment combination Ag NO_3 (100 ppm) + Sucrose 4% + Distilled water as holding solution was envisaged as the second best in extending the vase life of gerbera cut flowers viz., Sun Way, Dana Ellen and Rosalin. On other hand, a gift-wrapped (aqua pack) hand-tied design/floral arrangements are often with a holding solution below the binding point, covering the stalks could be an innovative idea, it is intended as a substitute for a vase to extend the freshness of flower [7]. Nowadays, professionals in floristry industry is very keen to improve the standards with respect to making of bouquets and floral arrangements, all their insight into traditional and modern methods to improve the quality and vase life of cut flowers to extended days. In line with above facts, the present investigation entitled "Effect of Aqua pack with holding solutions on quality and vase life of gerbera" was carried out to find desirable treatment for extending vase life under ambient room temperature.

Materials and Methods

Raw materials**:** Gerbera (*Gerbera Jamesonii)* Cv. Dana Ellen and aqua pack were collected from M/S, Gayathiri Farm, Hosur, Tamil Nadu, India.

Description of materials**:** The gerbera flowers are large in size, attractive yellow colour & capitulum is around 8-12 cm diameter as well as 50-75 cm long thick stalk and aqua pack is made from eco-friendly polyethylene (transparent) sheet with 50-100-micron thickness.

Pre-treatment**:** The flowers were harvested at perfect maturity stage and carefully brought to the laboratory without causing any damage and they were kept in sucrose (5%) for 2 hours as a standard pulsing treatment before the experiment.

Laboratory condition and holding solutions**:** The experiment was conducted in the Postharvest Laboratory, Department of Horticulture, Faculty of Agriculture, Annamalai University, India, during 2017-2018. It has 80-85 per cent average relative humidity in ambient room temperature under fluorescent lights (40 W) to maintain 12 hours of photoperiod. The holding solution comprising $AgNo_3$ @ 25 and 50 ppm, Benzyl Adenine @150 and 200 ppm and 8-HQS @ 200 and 300 ppm as individual treatments and in different combinations in this experiment.

Experimental design**:** This experiment was laid out in Completely Randomised Design (CRD) with three replications. Each treatment unit consisted of fifteen (15) flowers and with five (5) flowers representing a replication (Plate 1).

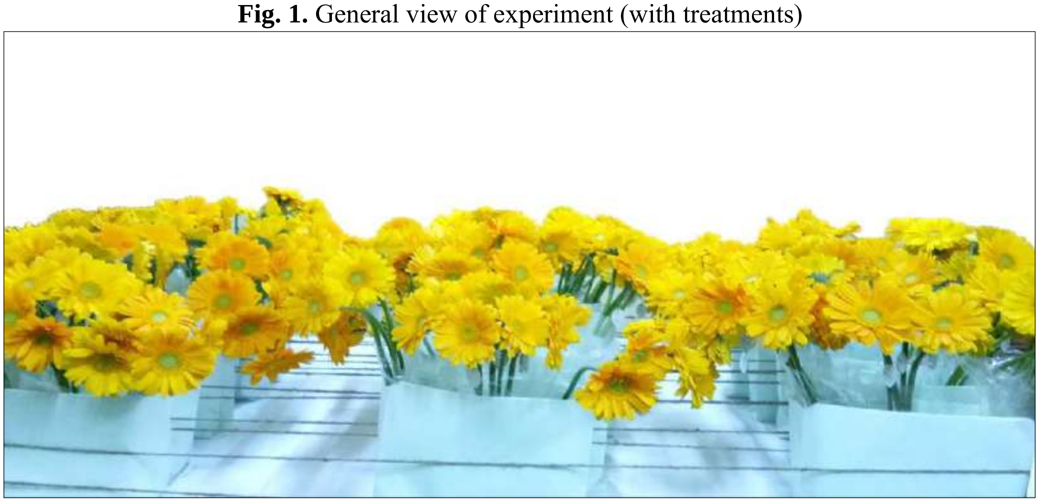
***Experimental treatments*:**

T_1-AgNo$_3$ @ 25ppm	T_{10}-AgNo$_3$ @ 25 ppm + 8-HQS @ 300 ppm
T_2-AgNo$_3$ @ 50 ppm	T_{11}-AgNo$_3$ @ 50 ppm + BA@150 ppm
T_3-BA @ 150 ppm	T_{12}-AgNo$_3$ @ 50 ppm + BA @ 200 ppm
T_4-BA @ 200 ppm	T_{13}-AgNo$_3$ @ 50 ppm + 8-HQS @ 200 ppm
T_5-8-HQS @ 200 ppm	T_{14}-AgNo$_3$ @ 50 ppm + 8-HQS @ 300 ppm
T_6-8-HQS @ 300 ppm	T_{15}-BA @150 ppm + 8-HQS @ 200 ppm
T_7-AgNo$_3$ @ 25 ppm + BA @150 ppm	T_{16}-BA @ 150 ppm + 8-HQS @ 300 ppm
T_8-AgNo$_3$ @ 25 ppm + BA @ 200 ppm	T_{17}-BA @ 200 ppm + 8-HQS @ 200 ppm
T_9.AgNo$_3$ @ 25 ppm + 8-HQS @ 200 ppm	T_{18}-BA @ 200 ppm + 8-HQS @ 300 ppm
T_{19}-Control (Distilled water)	

***Observations recorded*:** Flowers were kept for observation at quality and postharvest parameters *viz*., cumulative uptake of water (g^{-1}flower), cumulative transpiration loss of water (g^{-1}flower), physiological loss in weight, fresh weight of flower and PH of vase solution from 1st day to till the end of the vase life. The stem strength in gerbera was determined by angle between the main stem and stem just below the capitulum and they are classified based on four degrees of bending. The scape curvature was measured using a protractor and expressed in degrees [8], diameter of flower in cm, flower discolouration/fading days was assessed according to the procedure described by Macnish *et al*., [9] with rating scale of 1= none, 2 = slight fading and 3 = advanced fading and expressed in days. The vase life values were expressed in days and profitability of cost economics was calculated.

***Statistical analysis*:** The data were subjected to statistical analysis as per the procedure outlined by Panse and Sukhatme [10] and the results were tested at 5% level of significance.

Fig. 1. General view of experiment (with treatments)



Result & Discussion

Effect of aqua pack with holding solution on CUW, CTLW, CPLW and P^H of Vase solution in gerbera

The results recorded on cumulative uptake of water, cumulative transpirational loss of water, cumulative physiological loss in weight and PH of vase solution are presented in Table 1. In this experiment, with respect to water relations characters, lower stalks held in holding solution containing T_{10} ($AgNo_3$ @ 25 ppm + 8-HQS @ 300ppm) recorded the maximum cumulative uptake of water (35.68 g^{-1} flower), registered minimum transpiration loss of water (30.22 g^{-1} flower) and cumulative physiological loss in weight (18.65%) with a P^H of 0.84. Enhancement in water uptake by flower might to be due to the fact that silver nitrate present in holding solution acted as a biocide inhibiting microbial population that might have resulted in blockage of the vascular tissues. A similar result was observed by Acharya *et al*., [11]. Some of the studies revealed that 8-HQS alone or its combinations could increase holding solution uptake in cut flowers by preventing the growth of microorganisms in xylem and maintained water uptake of flower stems by Banaee *et al*., [12].

The ideal combination of holding solution might have contributed to reduced rate of respiration in flower petals resulting in lower cumulative transpiration loss of water. Similarly, Liao *et al*., [13] reported that water loss through transpiration was maintained at a stable level in the initial days and finally increase in water loss occurred before wilting in cut gerbera. Further, whenever the amount of transpiration exceeds absorption, a water deficit and wilting will develop.

This deficit will be reflected in corresponding reduction in water potential of plant tissues.

Table 1. Effect of aqua pack with holding solution on CUW, CTLW, CPLW and P^H of Vase solution in gerbera

Treatments	**CUW (g^{-1} flower)**	**CTLW (g^{-1} flower)**	**CPLW (%)**	**P^H of Vase solution**
T_1	30.10	32.37	43.02	6.98
T_2	28.78	32.00	56.87	7.41
T_3	27.35	33.37	45.08	7.56
T_4	27.58	33.70	43.06	7.32
T_5	32.09	30.78	27.85	5.96
T_6	34.65	30.62	18.65	3.82
T_7	28.41	32.41	39.79	7.29
T_8	28.36	32.60	38.39	7.01
T_9	30.15	31.43	21.97	5.53
T_{10}	35.68	30.22	16.71	3.86
T_{11}	27.74	33.18	42.91	7.56
T_{12}	28.64	32.56	29.82	5.92
T_{13}	30.84	32.24	23.88	5.37
T_{14}	32.93	31.51	20.68	5.42
T_{15}	28.59	34.51	38.22	7.03
T_{16}	28.92	34.47	26.71	6.81
T_{17}	29.90	34.17	25.98	5.72
T_{18}	30.51	34.63	24.35	5.83
T_{19}	25.50	36.86s	71.75	6.11
Grand Mean	**29.82**	**32.82**	**34.51**	**6.233**
SED	**0.16**	**0.15**	**1.34**	**0.12**
CD(P=0.05)	**0.33**	**0.30**	**2.69**	**0.26**

Effect of aqua pack with holding solution on fresh weight, stem strength and diameter of flower in gerbera

The results recorded on fresh weight of flower, stem strength and diameter of flower are presented in Table 2. All the treatments were found to be superior over control. The treatment, T_{10} ($AgNo_3$ @ 25 ppm + 8-HQS @ 300ppm) retained the maximum fresh weight (131.30 g^{-1} flower), highest resistance on stem strength (50.60) and maximum flower diameter at 11^{th} day after experiment. All these characters on different treatments showed a gradual reduction in the values at end of vase life. The aqua pack with holding solution (T_{10}- $AgNo_3$ @ 25 ppm + 8-HQS @ 300ppm) maintained fresh weight due to reduction in respiration transpiration rate and to check deterioration of cell ultra-structure as resulted out by Das *et al*., [14]. In this context, curvature was related to water potential of ray petals, increase in scape curvature depending on concentration of bacteria in water which blocks the xylem conducts leads to high resistance in stem strength and maintains flower diameter. This finding is in consonance with Soad *et al*., [2] in gerbera.

Table 2. Effect of aqua pack with holding solution on fresh weight, stem strength and diameter of flower in gerbera

Treatments	Fresh Weight (gm)		Stem Strength (degree)		Diameter of Flower (cm)	
	1^{st} day	11^{th} day	1^{st} Day	11^{th} Day	1^{st} day	11^{th} Day
T_1	127.60	96.60	77.96	35.25	11.60	8.88
T_2	123.60	83.30	75.32	32.23	10.80	8.24
T_3	126.60	94.00	76.00	32.48	10.95	8.45
T_4	127.60	94.60	76.43	34.00	11.00	8.34
T_5	133.30	111.60	80.75	44.35	11.38	9.45
T_6	137.80	130.60	88.96	50.60	11.87	10.1
T_7	130.00	99.30	79.00	37.57	11.18	9.06
T_8	131.60	107.30	79.66	38.12	11.19	9.20
T_9	137.00	124.30	86.54	49.36	11.75	9.90
T_{10}	138.00	131.30	89.60	54.65	11.63	10.17
T_{11}	129.60	98.30	78.01	35.57	11.17	9.02
T_{12}	132.60	108.60	80.63	42.47	11.36	9.35
T_{13}	136.50	121.60	85.89	49.30	11.59	9.81
T_{14}	137.50	125.30	88.38	49.76	11.84	10.05
T_{15}	132.30	108.00	79.68	40.91	11.22	9.28
T_{16}	135.80	112.30	82.00	44.41	11.45	9.60
T_{17}	136.00	114.00	83.40	48.05	11.49	9.68
T_{18}	136.30	116.00	83.42	48.63	11.53	9.74
T_{19}	117.00	76.30	69.00	31.34	10.37	8.16
Grand Mean	**133.17**	**108.06**	**81.05**	**42.05**	**11.35**	**9.27**
SED	**2.66**	**2.10**	**1.59**	**0.81**	**0.22**	**0.19**
CD(P=0.05)	**5.39**	**4.26**	**3.22**	**1.64**	**0.45**	**0.38**

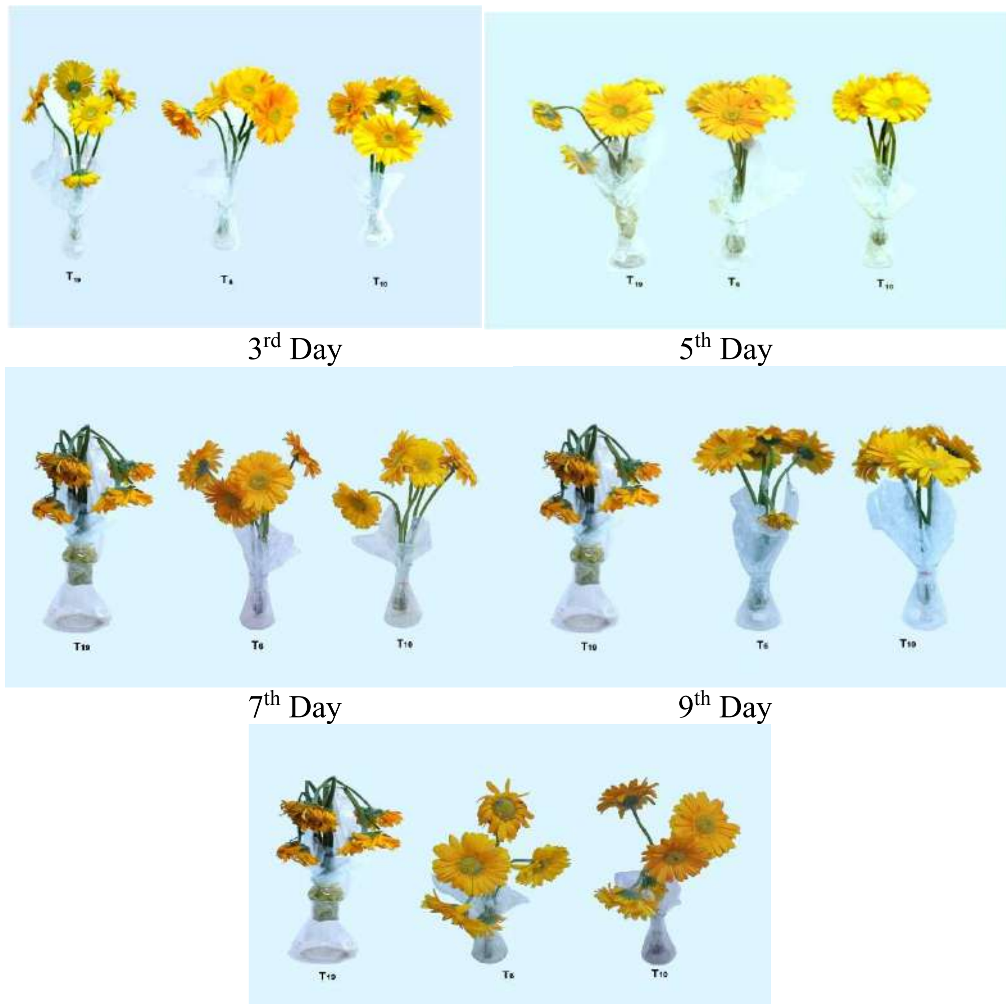
Effect of aqua pack with holding solution on flower discolouration, vase life, total cost of the treatment and vase life cost per day (ratio) in gerbera

The results recorded on flower discolouration, vase life, total cost of the treatment and vase life cost per day (ratio) are presented in Table 3. Among the treatments, gerbera flower stalks held in $AgNo_3$@ 25 ppm + 8-HQS @ 300 ppm resulted in obtaining longer vase life (11.95 days) whereas shorter vase life of flowers (5.97 days) was obtained by control (distilled water). The prolongation of vase life depended on maintenance of fresh weight, good water balance ratio, improved water uptake and low transpiration loss by Javad *et al*., [15]. The ethylene action inhibiting property of 8-HQS and Silver nitrate as a source of energy might have helped to get longer vase life of the cultivar of gerbera. These results are in conformation with Amith *et al*., [16] and Jafarpour *et al*.,

[17]. Overall, it was observed that aqua pack was very effective, might played an important role to maintain quality and enhance the vase life of gerbera Cv, Dana Ellen with irrespective of the treatment except control. During the course of experiment, it was found that growth of microbes in holding solution was inhibited due to presence of biocides. It has clearly indicated that significant influence over water relation characters and other postharvest qualities to vase life enhancement. These findings are in consonance with Lynda Owen [7] in gerbera as well as many cut flowers.

Table 3. Effect of aqua pack with holding solution on flower discolouration, vase life, total cost of the treatment and vase life cost per day (ratio) in gerbera

Treatments	Flower discolouration/fading (days)	Vase life (days)	Total cost (Rs.)	Vase life cost/day (ratio)
T_1	8.21	6.09	26.5	4.35
T_2	6.13	6.27	29	4.62
T_3	7.08	7.22	31.5	4.36
T_4	8.19	7.61	34	4.46
T_5	9.50	9.03	36	3.98
T_6	10.52	11.07	42	3.79
T_7	8.47	8.21	34	4.14
T_8	8.57	8.36	36.5	4.36
T_9	10.42	9.72	38.5	3.96
T_{10}	11.33	11.95	44.5	3.72
T_{11}	8.32	8.73	36.5	4.18
T_{12}	9.37	9.71	39	4.01
T_{13}	10.40	10.08	41	4.06
T_{14}	10.48	10.89	47	4.31
T_{15}	8.62	8.93	43.5	4.87
T_{16}	9.50	9.82	49.5	5.04
T_{17}	9.55	9.94	46	4.62
T_{18}	9.58	9.97	52	5.21
T_{19}	4.43	5.97	24	4.02
Grand Mean	**8.87**	**8.92**	-	-
SED	**0.17**	**0.10**	-	-
CD(P=0.05)	**0.35**	**0.21**	-	-



3rd Day 5th Day

7th Day 9th Day

11th Day

Fig. 2.

Conclusion

Analysing cost economics is most important to select a treatment for commercial adoption.

Hence, the treatments were subjected to economic analysis to arrive at conclusion for commercial application as suggested by Chandrashekar [18]. The treatment T_{10} ($AgNo_3$ @ 25 ppm + 8-HQS @ 300 ppm) has recorded the lowest total cost to vase life ratio of 3.47. with 11.95 days of useful shelf life followed by T_6: 8-HQS @ 300 ppm with cost to vase life ratio of 3.54. Hence, it is concluded that keeping the gerbera flowers in aqua pack with $AgNo_3$ @ 25 ppm + 8-HQS @ 300 ppm was considered as best option to adopt by commercial floral vendors (Fig. 2).

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Morphological and Physiological Characteristic of Three Varieties of Cut Chrysanthemum (*Chrysanthemum Morifolium* R.) Cultivated on Different Altitude in Indonesia

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Abstract

Chrysanthemums have been widely cultivated from the highland to medium land in Indonesia. The aim of research was to study the environmental effects of altitude between high land and medium land on the morphology and physiology of three chrysanthemum varieties viz Yatsatsuki, Arosuko Pelangi and Socakawani. Experiments at each location was arranged a Randomized Complete Block Design (RCBD) with three replications. Data were analyzed by combined ANOVA from two location data. The results showed that there were no interactions between the altitude and varieties of all parameters. The micro climate data showed that the medium land higher light intensity, higher air temperature, and lower relative humidity than in high land. High temperature and light intensity caused chrysanthemum had slower net assimilation rate (NAR) and crop growth rate (CGR) up to 10 weeks after plantating (wap). Furthermore, chrysanthemum in medium land had higher NAR at flowering time than in high land. Hence, chrysanthemum cultivated in medium land had longer of time of bud initiation, longer stem, smaller diameter, longer internodes, longer flower stalk, lesser number of flowers, longer flowering time and paler red cooler than in high land. Therefore, all of the varieties can be developed in the medium land but it was needed improved cultivating technology to get the quality as well as in the high land.

Keywords: chrysanthemum, high land, medium land, morphology, physiology, quality

Introduction

Chrysanthemum has been developed and adapted in Indonesia where high land areas around 1000 meters above see levels is most suitable places [1]. For while, chrysanthemum business is dominated by urban community in medium land to low land [2]. Intensification area of chrysanthemum in the medium land is one of the strategies to increase production of chrysanthemum [3]. Hence, the production of chrysanthemums in the medium land can accelerate the chain distribution to reach the consumer. The medium land has more light intensity and higher air temperature than those of in the high land. As the consequence, cultivation of chrysanthemum in medium land might change the characters of plant growth and flower quality [3]. The effect of unfavorable conditions for cut chrysanthemum had been reported in the other country such us late open flower [4], lower diameter of flower [5], lower intensity of flower color [6]. This caused decreasing in the quality of cut chrysanthemum because high quality was determined by height of plant, number of flowers, size of flower, and color of flower [7]. In cut chrysanthemum this change was influenced by variety [8]. Most of chrysanthemum varieties that are breaded by Indonesian government are only adapted to high land. For while, location of chrysanthemum cultivated is not only in high land but also in medium land. In fact, there are many varieties with flower colors such us white, yellow and red which dominates the chrysanthemum market [9]. Some of these varieties might be potential to be cultivated in medium land such us Yatsastuki, Arosuko Pelangi and Socakawani which have white, yellow, and red petal respectively. However, we did not know the changes in quality when some of these varieties was planted in the medium land. The objectives of the research to study environmental effects i.e., light intensity, air temperature and relative humidity between high land and medium land to the change of physiological and morphological characters chrysanthemum varieties. It can be used to determine varieties that are developed in the medium land.

Materials and Methods

Research procedure

The research was conducted from December 2017 to March 2018. The spray type *Chrysanthemum morifolium* cv. Yatsatsuki, Arosuko Pelangi and Socakawani rooted cuttings (7 cm long with 3-4 leaves) were used as plant materials. Rooted plants then transferred to be cultivated in the screen house in the high land (1100 m asl) in Cipanas, West Java and the medium land (485 m asl) in Samigaluh, Yogyakarta, Indonesia. The soil media from Kulonprogo (medium land) was used as planting media both in high land in medium land. The plants were maintained based on Operational Standar Prosedure of chrysanthemum cultivation in Indonesia [10].

Research design

There were two factors in this experiment. The first factor was the cultivation location in the high land and the medium land and the second factor was the chrysanthemum varieties viz. Yatsatsuki (white petal), Arosuko Pelangi (yellow petal) and Socakawani (red petal). The experimental design at each location was arranged by using a Randomized Complete Block Design (RCBD) with three replications.

Measurement of observation parameters

The microclimate observation were environmental temperature, relative humidity, and light intensity were average weekly data. The data were monitored every day at 07.00 am, 09.00 am, 12.00 pm, 15.00 pm, and 17.00 pm.

The growth response such as (1) Net Assimilation Rate (NAR) = $((W_2\text{-}W_1)/(t_2\text{-}t_1))$ x $((\ln LA_2\text{-}\ln LA_1)/LA_2\text{-}LA_1)$. (2) Crop Growth Rate (CGR) = 1/PD x $(W_2\text{-}W_1)/t_2\text{-}t_1$. Each organ such us root, leaf, stem, flower was dried at 70 °C until constant weight. The information of the symbols was LA_2 = leaf area at the time of observation, LA_1 = leaf area in the previous observation, PD = plant density, W_2 = total dry weight at time of observations, W_1 = total dry weight in previous observations, t_2= time of observation, t_1=time previous observation. Leaf area was measured using ImageJ software program (ImageJ, Maryland, USA). These parameters were monitored on 2 weeks after plantating (wap), 5 wap, 10 wap, and flowering time. The external quality traits parameter observed were (1) the initiation of flower buds, (2) the height of plant, (3) the diameter of the stem, (4) the length of internodes (5) the height of peduncle, (6) the number of flower plant was number of blooming flower plus flower bud up to 20 cm, (7) the number of blooming flower (8) the diameter of the flower, (9) the flowering time, (10) the intensity of color of the flower (*L*, *a, b* value) using chromameter (Kinoka Minolta, Japan. These parameters were monitored on flowering time.

Statistical analysis

The data were analyzed used combined ANOVA from two location data. The Tukey test with alpha 5% was applies if there was significantly different. In addition, the micro climate data of high land and medium land were analyzed by T test alpha 5%. All statistical analyses were performed using SAS.

Results

Micro climate parameters

The average of light intensity, air temperature and relative humidity in the high land significantly difference with in the medium land (Table 1).

Table 1. Micro climate observation

	Light intensity (lux)				Air temperature (ºC)				Relative humidity (%)			
	1	2	3	average	1	2	3	average	1	2	3	average
HL	17259	15810	16965	16678	24.88	23.46	23.92	24.1	80.61	82.33	80.53	81.2
ML	22701	27324	29235	26420	28.58	29.04	30.21	29.3	79.69	75.39	73.78	76.3
	*	*	*	*	*	*	*	*	ns	*	*	*

Noted: HL=high land, ML= medium lan, 1= 0-5 wap; 2= 6-10 wap, 3=11-15 wap
Mean followed the same letter at the same column are not significantly difference based on uji T, p=0.05. Sign (ns) showed there was not significance and sign (*) showed there was significance between highland and medium land.

Growth responses

There was no interaction between altitude and varieties to growth response. Chrysanthemum in medium land had slower NAR and CGR at 5-10 WAP than in high land. But there was significantly different NAR and CGR 10 wap up to flowering between medium and high land (table 2).

Table 2. Growth responses of chrysanthemum cultivated between high land and medium land

Treatments	5 wap		10 wap		Flowering time	
	Net assimilation rate (g.cm^{-2} d^{-1})	Crop growth rate (g.g^{-1} d^{-1})	Net assimilation rate (g.cm^{-2} d^{-1})	Crop growth rate (g.g^{-1} d^{-1})	Net assimilation rate (g.cm^{-2} d^{-1})	Crop growth rate (g.g^{-1} d^{-1})
Location						
High land	0.00076 a	0.0014 a	0.00052a	0.0037 a	0.000159 a	0.0225 a
Medium Land	0.00062 b	0.0014 a	0.00033b	0.0022 b	0.000205 a	0.0221 a
Varieties						
Yatsatsuki	0.00072 a	0.0015 a	0.00037 b	0.0025 b	0.00010 b	0.012 b
Arosuko Pelangi	0.00063 a	0.0013 a	0.00043 ab	0.0026 b	0.00022 a	0.022 ab
Socakawani	0.00073 a	0.0017 a	0.00047 a	0.0037 a	0.00022 a	0.033 a
CV (%)	*14.10*	*17.61*	*11.42*	*9.89*	*30.98*	*34.19*
interaction	*ns*	*ns*	*ns*	*ns*	*	*

Mean followed the same letter at the same column are not significantly difference based on Tukey Test, p=0.05. Sign (ns) showed there was not interaction and sign (*) showed there was interaction between altitude and varieties. Sign (-) showed data could not be observed.

External quality traits

There was no interaction between altitude and varieties to external quality traits. Different altitude influenced changing of all parameter's morphology except number of flower and *b* value of petal flower. The chromacity value in each variety showed in accordance with the color variations of each chrysanthemum variety (table 3).

Table 3. External quality trait of chrysanthemum cultivated between high land and medium land

Treatetment	Initiation of flower bud (day)	Height of plant (cm)	Diameter of stem (cm)	Length of internodes (cm)	Length of peduncle (cm)	Number of flowers	Number of blooming flowers	Diameter of blooming flower (cm)	Flowering time (day)	Colour of petal *L*	*a*	*b*
Altitude												
HL	47 b	100.14 b	6.95 a	3.07 b	13.85 b	11.15 a	9.17 a	5.69 a	92.43 b	64.70 b	14.18 a	29.47a
ML	48.37 a	128.81 a	6.07 b	3.64 a	18.99 a	11.16 a	5.32 b	4.69 b	106 a	70.02 a	10.52 b	33.50a
Varieties												
Y	47.52 a	114.06 a	5.76 b	3.49 a	15.62 a	11.04 a	7.56 a	5.82 a	98.0 c	91.47 a	3.25 b	2.575 a
A	47.76 a	112.56 a	6.21 b	3.25 b	15.94 a	10.02 a	6.75 a	4.66 b	99.0 b	79.12 b	-0.183 b	73.71 a
S	47.78 a	116.79 a	7.55 a	3.36 ab	17.70 a	12.41 a	7.43 a	5.09 b	100.0 a	31.56 c	33.98 a	18.16 b
CV (%)	*6.03*	*4.73*	*8.14*	*4.06*	*8.00*	*15.06*	*9.21*	*7.42*	*0.00*	*3. 40*	25.20	9.38
interaction	*ns*	*ns*	*ns*	*ns*	*ns*	*Ns*	*ns*	*ns*	ns	*ns*	*ns*	*ns*

Note HL= highland, ML= medium land, Y= Yatsatsuki, A= Arosuko Pelangi, S= Socakawani
Mean followed the same letter at the same coloumn are not significantly difference based on Tukey Test, p=0.05.
Sign (ns) showed there was not interaction and sign (*) showed there was interaction between altitude and varieties.

Discussions

The increasing of the temperature and light intensity in medium land at 0-5 wap (vegetative phase) caused NAR in medium land was lower than in the high land. The NAR of chrysanthemum increased when it was exposed to the light intensity between 171-485 μmol^{-2}s^{-1} (12141-34435 lux) at temperature was 24 °C furthermore, the NAR was decreased when temperature above 28 °C [11].

At 6-10 wap, light intensity, temperature, and relative humidity in medium land were 27324 lux, 29.04 °C, 75.39% respectively. Optimal light intensity for flower development was ± 20191 lux [12] up to 32000 lux [13] with optimal temperatures of 18-23 °C [14]. Temperatures above 23 °C increased respiration. Respiration used the substrate from photosynthesis, it caused reducing photosynthesis [15]. The lower NAR at 5 wap indicated that fewer starch reserves in the leaves for the formation flower buds [16]. That resulted the flower bud initiation in the medium land was 2 days slower than the high land. Furthermore, flower development phase, the growth of chrysanthemum stems planted in the medium land was faster than in high land with longer internodes and smaller diameter. Because of this condition, the rate of flowering in the medium land was delayed for 14 days (Table 3). High temperatures increased the rate of respiration in the flower buds, it caused flower buds lack of energy to expand cell and decreased in the speed of flowers blooming [17]. Lower *a* value showed red petal chrysanthemum cultivated in medium land fader than in high land and it showed decreasing in anthocyanin content [18]. But, the same *b* value showed that different altitude did not influenced carotenoid content [19].

Conclusion

High temperature and light intensity caused chrysanthemum have slower NAR and CGR until flower bud stadia furthermore chrysanthemum in medium land had higher NAR at flowering time.

As the consequence, chrysanthemum varieties cultivated in the medium land have a longer stem, smaller diameter, longer internodes, longer flower stalk, less number of flowers, longer flowering time, paler red color than in high land. Therefore, all of the varieties can be developed in the medium land but it is needed improved technology cultivated in order the quality as well as in the high land.

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IMPLEMENTATION OF INTEGRATED CROP MANAGEMENT TO SUPPORT FOOD SOVEREIGNTY BASED ON BIO-INDUSTRY

Increasing Genetic Diversity and Phenotype Response of Torbangun (*Coleus amboinicus* Lour.) Through Gamma-Ray Irradiation

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Abstract

The diversity of Indonesian *Coleus amboinicus* Lour species is still needed to be increased.

This species was called a torbangun plant by the Batak tribe in Indonesia. It has been known to stimulate breast milk production. The research was performed to increase the genetic diversity of torbangun by using gamma-ray irradiation. The experiment used five levels of gamma-ray in Randomized Complete Block Design (RCBD) with three replication per treatment level. The nodes of torbangun were cultured on *in vitro* MS medium supplemented with 1.5 mg/l 6-Benzylaminopurine (BAP) and 1 mg/l Gibberellic Acid (GA3) to produce *in vitro* shoots, after the shoots produced the roots, the plantlets were irradiated with gamma-ray at the dosage of 10, 20, 30, 40, and 50 Gray. Irradiated plantlets were transferred to fresh MS medium supplemented with GA3 and BAP. The results showed the Lethal Dose 50 (LD_{50}) of gamma-ray irradiation on torbangun plantlets was at 44.036 Gray. The phenotypic coefficients of variation were relatively higher than genotypic ones on plantlet height, the number of leaves, shoots, and roots. Gamma-ray irradiation could produce putative torbangun mutants with the variation on leaf color such as purple, variegated green, variegated yellow and dark green while some plantlets changed to be albino.

Keywords: gamma-ray, leaf color, mutant, plantlets, variation

Introduction

Coleus amboinicus Lour is one of a herbaceous plant of the Lamiaceae family. It has a fragrant aroma like fresh oregano. The stem structure is fleshy, branched and hairy. The leaves are oval, wide, and thick. The flowers are short-stemmed and purple in a dense collection [1]. It's vegetatively propagated and the suitable planting area is in a slightly shady environment. This plant is known as the torbangun plant among the Indonesian people, especially in the Batak tribe, North Sumatra. Today, torbangun is more well-known as an herbal plant that have function to stimulate breast milk production [2, 3, 4, 5], to decrease a systolic blood pressure [6], to decrease a cholesterol level [7] and to reduce the complaints of pre-menstrual syndrome (PMS) [8]. Besides as herbal plant, this plant has the potential as an ornamental plant. In low land, flowers are difficult to form, but the leaves still grow dense, clustering like a beautiful flower bouquet. The stem can grow creeper, therefore it can be used for fencing. In addition, torbangun reported as one of world economic plants that important for international commerce [9].

There are many ornamental plants in nature that have beautiful color and shape of flowers, stems, and leaves. This plant belongs to the genus of *Coleus* which has been widely known in floriculture as an ornamental plant. One famous species of *Coleus* with many varieties of its leaf shapes and colors is *C.blumei* [10]. In contrast to *C. blumei*, *C. amboinicus* have one leaf color, which is green.

The genetic diversity of torbangun is still in the low category. Genetic diversity of torbangun is needed to be done, both for herbal germplasm and its aesthetic value as ornamental plant.

Genetic diversity improvement for torbangun plants had been carried out using gamma-ray irradiation on shoot cuttings [11, 12] and chemical mutations [13]. The results showed the difference between mutant and wild-type plants in green and leaf shape. Meanwhile, other *Coleus* such as *C. blumei* which have a variety of color changes in their leaves with gamma-ray irradiation [14].

Based on previous studies, the color change of the torbangun mutant for the green color pattern, have been obtained [12]. This treatment could be re-treated by gamma-ray irradiation on different plant materials such as torbangun plantlets. Torbangun plantlets obtained from *in vitro* culture as irradiation materials can increase the number of mutants obtained. This due to the in vitro mutagenesis method that could eliminate chimera. Chimera is the tissue or organ that have sectors of different cell genotypes, which show differences in phenotypes such as variance [15].

The study aimed to (1) obtain LD_{50} value of gamma-ray irradiation to induce mutation of torbangun plantlets, (2) study the effect of the gamma-ray irradiation on genetic diversity and phenotypic response of torbangun, and (3) obtain a new line of torbangun both for health and economic potential as ornamental plants by selection of mutants.

Materials and Methods

The study was carried out from November 2017 to August 2018 at the Tissue Culture Laboratory 1 Faculty of Agriculture, Bogor Agricultural University. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications. The plant material was plantlets of *Coleus amboinicus* Lour irradiated at PAIR (Center for Isotope and Radiation Applications), Pasar Jum’at – Jakarta. The irradiation source was gamma rays from Cobalt 60 through a *gamma cell* GC220 irradiator.

Research Phase

Developing of the In Vitro Population

The plantlets population were resulted from shoot culture of torbangun which come from the shoot tip. Sterilization of explants were started by washingg the explants with detergent for 4 hours, then rinsed with running tap water for 30 minutes. In the next step, explants were soaked in distilled water supplemented with 6 drops of tween 20 per 100 ml of distilled water, then explants were brought to Laminar Air Flow Cabinet (LAFC), transferredd subsequently into 5, 10 and 15 ppm of Clorox for 5 minutes in every Clorox concentration, then fungicide 30 g/100 ml, and alcohol 96% for 5 minutes After all above steps, explants were rinsed with sterile water for 3 times, then planted in MS medium supplemented with 1.5 ppm BA and 1 ppm GA3 for 2 weeks.

For shoots multiplication, explants were transferred to MS medium without plant growth regulator and sub-cultured for every 3 weeks, to get around 600 plantlets.

Induction of Mutations with Acute Irradiation

The population of torbangun plantlets with the vigorous organ were selected for irradiation.

The total of 525 plantlets were irradiated with 105 plantlets per dose, while the plantlets control was not irradiated. The doses were used for acute irradiation on 10, 20, 30, 40 and 50 gray (Gy).

Plantlets Subculture after Irradiation

Irradiated plantlets were subcultured into post-irradiation recovery medium (MS medium without plant growth regulator) for one week. Then, the plantlets were subcultured to shoot formation medium using BA 1.5 ppm + GA_3 1 ppm as the first generation of mutants or MV1 (Vegetative Mutant 1) and stored in the culture room for a month. Survive plantlets which produced new shoots were subcultured (into MV2 generation) to shoot formation medium containing 1.5 ppm of BA. Care and prevention of contamination were done by spraying 70% alcohol around bottles and culture shelves every week. The contaminated plantlets were separated from sterile plantlets.

Data Analysis

Data scoring was conducted weekly starting one week after subculture. The data measured and calculated for LD_{50}, plant height (cm), number of leaf, root and shoot. Qualitative characters such as leaf colour was observed using RHS mini color chart to identify plant phenotype response after gamma-ray irradiation.

Data from the MV1 generation were analyzed using SAS 9.0 application with Duncan's follow-up test and MV2 data were analyzed by qualitative Box-plot using the Minitab series 16.0 application. Determination of LD_{50} used the Curve Expert 1.3 software. Estimating genetic parameter included the coefficient of phenotype variation (PCV), the coefficient of genotype variation (GCV) [16] and broad sense heritability (h^2bs) [17] were analyzed with Microsoft. Excel.

$$PCV\% = \frac{\sqrt{\sigma^2 p}}{\bar{\chi}}\ x\ 100\%$$

$$GCV\% = \frac{\sqrt{\sigma^2 g}}{\bar{\chi}}\ x\ 100\%$$

$$h^2bs = \frac{S_1 - S_0}{S_1}\ x\ 100\%$$

Description:

σ^2_p=phenotype variance

σ^2_g=variance of genotype

S_0=variance among clones

S_1=Variety of irradiation derivatives

$\bar{\chi}$=average of population

The dendrogram rectangle type with dissimilarity Gower method and average linkage clustering method using PBSTAT-CL 1.7 was used for diversity analysis.

Result and Discussion

Lethal Dose 50 (LD_{50})

LD_{50} is a gamma-ray irradiation dose which causes 50% of plantlets death. The higher diversity from irradiation is predicted at around LD_{50} [11, 18]. The results of this experiment showed that the LD_{50} value was at 44,036 Gy (Table.1). This result was different from the value of LD_{50} in torbangun shoots mutation with gamma-ray irradiation in the previous research which the LD_{50} was 37.62 gray [11] irradiation material. Torbangun plantlets survive from 10 and 20 Gy were higher than that from other doses.

Table 1. Radio sensitivity level of torbangun plantlets based on Curve Expert analysis

Lethal dose	Model	Coefficient Data	Score (Gy)
LD_{50}	Quadratic fit $y = a + bx + cx^2$	a = 101.10 b = -0.06 c = -0.024	44.036

The number of survived plantlets torbangun was decreased at doses of 30 to 50 Gy. Plantlets that were died due to irradiation showed dry and rigid symptoms. Symptoms of the plantlet death were observed two weeks after irradiation. The number of doses affected the mortality rate. The higher level of irradiation dose, the higher the number of plantlets deaths. The parts of survived plantlets at doses of 40 Gy and 50 Gy were grown into new plantlets even though the parent of plantlets were dry and stiff for four weeks. The number of plantlets survived around the LD_{50} dose was 39% for a dose of 50 Gy and 52% at a dose of 40 Gy (Fig. 1). In addition, wild-type plantlets showed a vigorous growth with a percentage of life reached 100%.

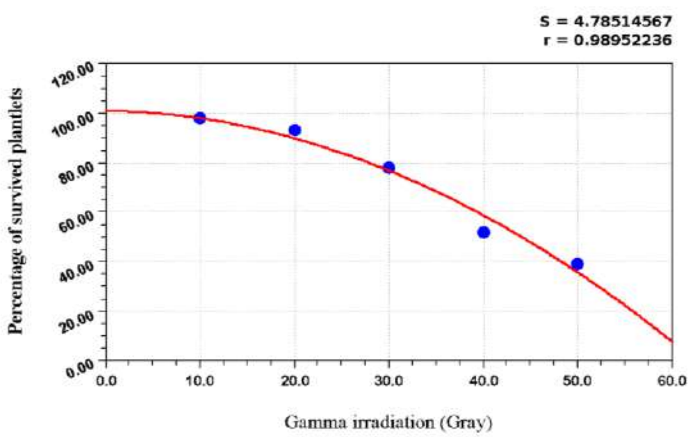

Fig. 1. Percentage of *Coleus amboinicus* plantlets survived at 8 weeks after irradiation

The Phenotype of Plantlet Torbangun at MV1 Generation

Early generation mutants (MV1) showed abnormal growth. The plantlet torbangun experienced a quantitative decrease in vegetative characteristics. The number of irradiation doses affected the differences in the quantitative trait of plantlets morphology. Increasing dosage caused the morphological quantity of the plantlet to decrease. The mutant morphology decreased in quantitative trait compared to wild-type plantlets, the trait was the height of plantlets, and the number of leaves, shoots, and roots. The four mutant traits were significantly different at the 5% level of wild-type plantlets and in all irradiation doses (Table 2).

Wild-type plantlets could grow well while irradiated plantlets experience a quantitative decrease in vegetative traits. The number of irradiation doses affected the quantitative trait differences of plantlets morphology. Higher the dose of irradiation given, causing the decreasing number of morphological quantities. The quantitative trait of the mutant morphology decreased compared to wild-type in all the traits observed, namely the trait of plantlets height, number of leaves shoots, and roots. The four mutant traits were significantly different at the 5% level with wild-type plantlets at all irradiation doses (Table 2).

The height of plantlet experienced a reduction in growth at all irradiation doses. The higher of dose causing a higher growth reduction (Table 2). This showed that the presence of irradiation doses affected the growth of plantlets. The same thing also was found in the number of shoots, roots and the number of leaves that decreased quantitatively because of the higher dose of irradiation. Doses of 10, 20, 30, 40 and 50 Gy could reduce the quantity of four traits observed.

These showed that phenotype responses of plantlet torbangun after gamma irradiation can significantly affect the quantitative variable of morphology. Unlike the case with the number of leaves at 10 Gy doses does not have a real difference with the number of leaves on wild-type plantlets.

Table 2. Average plantlet height, and the number of leaves, shoots, and roots plantlet torbangun of generation MV1

Trait	Dosage of gamma irradiation (Gy)					
	0	10	20	30	40	50
Plantlet height	2.215a	0.727b	0.427cb	0.174c	0.265c	0.294c
Number of leaves	10.669 a	8.372a	4.495b	1.924cb	0.581c	1.029c
Number of shoots	3.743a	1.180b	0.571cb	0.295c	0.133c	0.076c
Number of roots	2,039a	0.333b	0.171b	0.086b	0.019b	0.028b

Numbers followed by the same letter in the column and the same trait was not significantly different based on Duncan's test at 5% level.

The Phenotype of Plantlet Torbangun at MV2 Generation

The diversity on quantitative variable increased in the second generation (MV2) and high variation on phenotype response of new irradiated torbangun plantlets (Fig. 2). At the trait of plantlet height, there are many outliers at all doses except the 50 Gy dose. There was the highest plantlet outlier data in the treatment dose of 20 Gy compared the four other dose levels. The highest of plantlet diversity in the treatment dose of 10 Gy and 20 Gy which was observed from a large number of outliers. Similar to the number of shoots and roots, the highest variety and number of roots was found in the treatment of 10 and 20 Gy. Unlike the case with the number of leaves that had little diversity in all irradiation doses. The highest number of leaves was only found at a dose of 20 Gy.

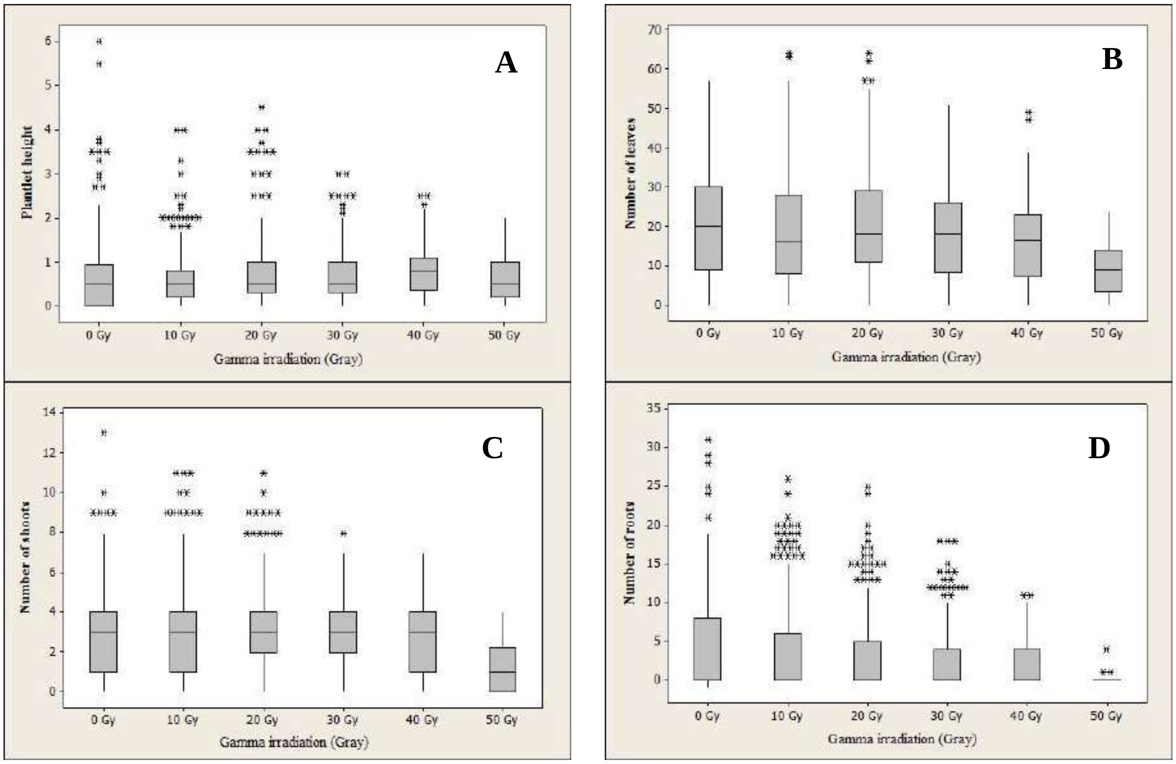

Fig. 2. Boxplot analysis of plantlet height (A), number of leaves (B), number of shoots (C) and number of roots (D) of plantlets of MV2 generation

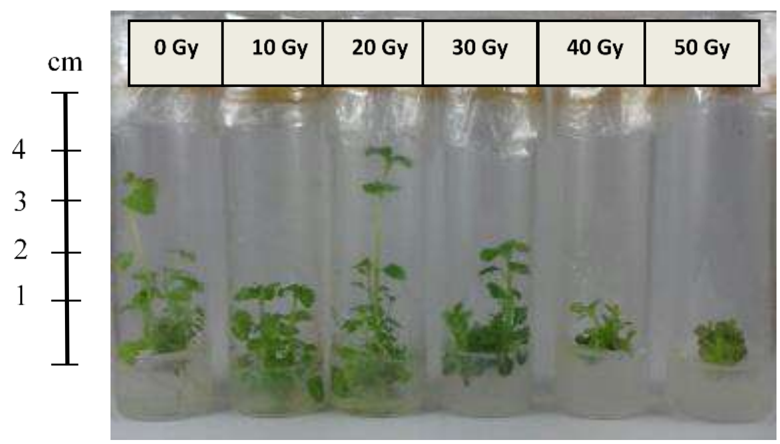

Fig. 3. The difference in height of plantlet torbangun at MV2 generation

The trait of the plantlets around the LD_{50} dose at 40 Gy did not produce the amount of diversity as much as those at dose 10 and 20 Gy. Giving a dose of 40 Gy had a diversity in the trait of plantlets height, number of leaves and roots, while the number of shoots did not have any diversity seen from the number of outliers. Plantlets with doses above LD_{50} have little diversity. The treatment of 50 Gy dose showed that it only has diversity in the number of root traits. The appearance of the torbangun plant height was observed to decrease with increasing irradiation doses (Fig. 3). A decrease in shoot length also occurred in *in vitro* potato plants due to physical irradiation treatment [19]. Similarly, cowpea plants experience a decrease in shoot and root length at the highest dose of gamma-rays. This occurred because of the reduction in mitotic activity in plant tissues [20].

Phenotype of Plantlet Mutant Color

Measurement of phenotype performance on leaf color level used RHS mini color chart at MV2 generation. The color of wild-type plantlet leaves was same as the color of the torbangun cutting leaves, RHS 137C Green. Based on the observations, there were putative mutant plantlets which had color changes on the leaves (Table 3). The doses at 10 Gy and 20 Gy produced the most of the leaf color putative mutants in purple, green variegata, yellow/green, dark green and albino (Fig 4). These showed that gamma irradiation can increase the diversity in the leaf color of the plantlets torbangun, especially at low doses.

In general, the leaf color of flowers was influenced by flavonoids and carotenoids. Flavonoids, as the same group of anthocyanins, play a role in producing yellow, blue, purple, red, etc.

Carotenoids are spread throughout the organ as important photosynthetic compounds [21]. The 40 Gy dose that was around the LD_{50} produced two putative mutants of dark green. The dose of 50 Gy also produced a dark green mutant. The color of albino was also produced on plantlets by dose of 30 Gy. Color changes in mutants occurred in some areas of the plantlet, both albino and other colors such as purple on the leaves. Albino only occurs in part of the plantlet organ from a bunch of new plantlets produced from one parent. Similarly, the purple color on the leaves was only found on some leaves at the upper part of the plantlet. This is presumably because the chimera, which is an individual composed of two or more different idiotypes, shows the cell channels in the apical meristem. Seeing the color produced on the leaves, allegedly including the type of mericlinal and periclinal chimera namely mutations that occur in the cell membrane and corpus [15].

Table 3. The leaf color of torbangun plantlet resulted from gamma-ray irradiation treatment at MV2 generation

Dose	Accessions	Leave color	RHS mini color chart
0 Gy	Wild-type	Green	RHS 137C Green
10 Gy	HT14	Purple	RHS 185DDark purple red
	HT11	Green variegated	yellow-green RHS 145A/RHS 141A dark green
	HT12	Green variegated	RHS 145A yellow-green/RHS 141A dark green
	HT13	Yellow/Green	RHS149A light yellow-green
20 Gy	HT22	Green	RHS 145A yellow-green/RHS 141A dark green
	HT23	Dark green	RHS 144A dark green
	HT24	Dark green	RHS 144A dark green
	HT21	Green/yellow variegated	RHS 2C light yellow-green/RHS 144A dark green
	HT25	Albino	RHS 157B white
	HT20	Yellow/Green	RHS 149A light yellow-green
30 Gy	HT31	Albino	RHS 157B white
	HT30	Albino	RHS 157B white
40 Gy	HT40	Dark green	RHS 144A dark green
	HT41	Dark green	RHS 144A dark green
50 Gy	HT50	Dark green	RHS 141A dark green

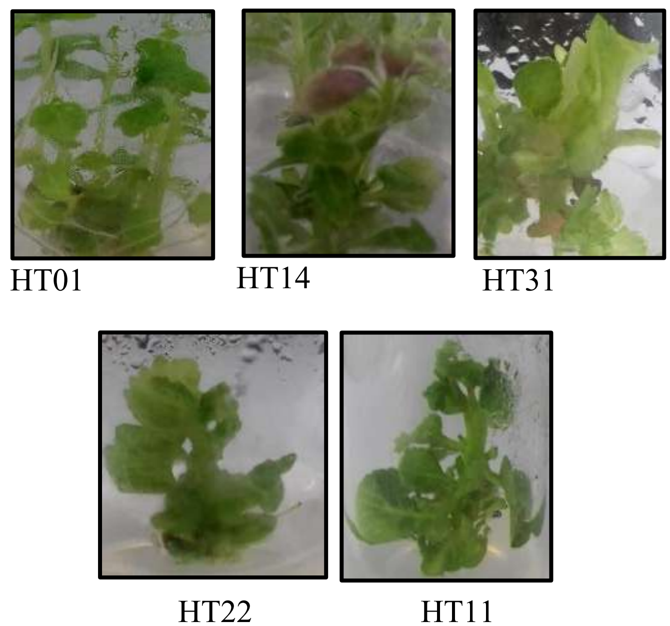

Fig. 4. The appearance of the leaf color of plantlets resulted from irradiation gamma-ray on MV2 generation. The HT01 as control (0 Gy); HT14, HT11 (10 Gy); HT22 (20 Gy) and HT31(30 Gy).

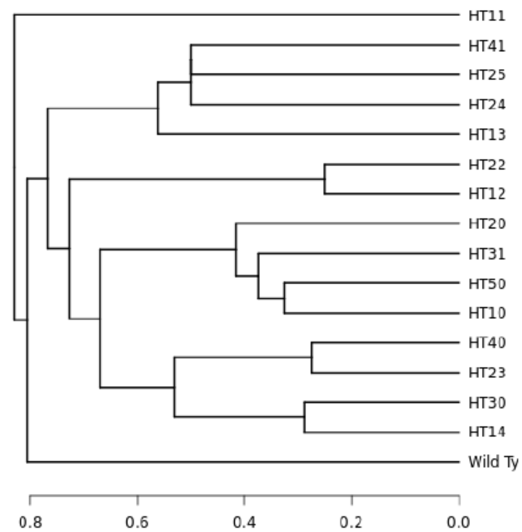

Fig. 5. The dendrogram of leaf color plantlet mutant of MV2 generation.

Based on the results of leaf color observation, 16 accessions were selected for analysis with a dendrogram of observed quantitative trait data. The dissimilarity level between genotypes had a difference between 0.0-0.8. The results of the dendrogram analysis showed that the HT14 was very closed to the wild-type, while the HT11 was appeared very distant. The farthest and closest distance of the mutant accessions with wild-type were from the mutant 10 Gy irradiation. The second farthest distance from the dose of 40 Gy was the HT41. Overall, there were 6 mutants that had the most distinction toward the wild-type (Fig. 5). The higher the difference in similarity between accessions, the higher the phenotype differences in accessions morphology. Cluster analysis such as dendrogram can be used to analyze a genotype of morphological traits and groups based on similarity [22].

Genetic Parameters of Plantlet In Vitro Torbangun

Analysis of genetic parameters (Table 2) on the MV2 generation of torbangun plantlets showed that the four morphological traits of torbangun had a high phenotypic coefficient of variation (PCV), except for the number of roots at a dose of 40 Gy. This proved that the quantitative appearance of the morphology of plantlets resulting from mutations had high phenotypic diversity.

Genetic diversity was also high in plantlets height with doses of 20, 40 and 50 Gy observed from the genotypic coefficient of variation (GCV). The number of shoots also had high genetic diversity values at doses of 10, 40 and 50 Gy. Different from the case in the number of roots and leaves had low GCV values in all doses of irradiation. This showed that there was no genetic diversity in the number of leaves and number of roots, although phenotypes look different and varied. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all observational traits. These indicated that the influence of the environment was very large on the phenotypic expression on the trait of observation [23].

The genetic diversity influenced the heritability value of the quantitative traits observed.

Determination of the selected trait based on the value of heritability. The classification of heritability values was divided into three criteria, low, moderate and high. Heritability was expressed, high ≥50%, moderate on 20-50%, and low ≤20% [24]. The results of the analysis showed a high heritability value only at the trait of the plantlet height, irradiated by 50 Gy. The moderate heritability value was obtained by the trait of the plantlet height in doses of 20 and 40 Gy, as well as the number of shoots at doses of 10, 40, and 50 Gy.

Table 4. Estimation of genetic parameter values of torbangun plantlets in the MV2 generation

Parameters Genetic	Dose (gray)	Phenotypes Variations		Genotype Variations		H^2bs	Criteria of Diversity
		σ^2 P	PCV (%)	σ^2 G	GCV (%)		
Plantlet Height	10 Gy	0.08	62.21	0.00	0.00	0.00	low
	20 Gy	0.15	69.15	0.04	35.52	26.38	moderate
	30 Gy	0.12	55.20	0.01	15.56	7.95	low
	40 Gy	0.20	94.63	0.09	62.34	43.39	moderate
	50 Gy	0.30	86.41	0.18	67.93	61.81	high
Leaves number	10 Gy	79.13	49.03	0.00	0.00	0.00	low
	20 Gy	67.36	46.08	0.00	0.00	0.00	low
	30 Gy	41.95	48.40	0.00	0.00	0.00	low
	40 Gy	60.16	61.93	0.00	0.00	0.00	low
	50 Gy	50.96	74.96	0.00	0.00	0.00	low
Shoots number	10 Gy	1.99	45.65	0.67	26.42	33.48	moderate
	20 Gy	1.35	31.18	0.03	4.32	1.92	low
	30 Gy	1.29	48.05	0.00	0.00	0.00	low
	40 Gy	1.97	55.20	0.65	31.66	32.89	moderate
	50 Gy	1.69	86.68	0.37	40.32	21.64	moderate
Roots number	10 Gy	0.50	47.14	0.00	0.00	0.00	low
	20 Gy	4.50	84.85	0.00	0.00	0.00	low
	30 Gy	0.33	43.30	0.00	0.00	0.00	low
	40 Gy	0.00	0.00	0.00	0.00	0.00	low
	50 Gy	3.00	86.60	0.00	0.00	0.00	low

Phenotype variance (σ^2P), Genetic variance (σ^2G), the phenotypic coefficient of variation (PCV), the genotypic coefficient of variation (GCV), broad sense heritability (H^2bs).

The quantitative trait of the number of leaves and the number of roots had a low heritability value because it did not have the value of genetic diversity. The low heritability for these traits showed that direct selection will be ineffective [23], especially for torbangun plantlet. The results of variance 0 value showed that there was no genetic diversity in the morphological trait (Table 4), although phenotypically different. The low heritability value explained that the environment had a big influence on plant growth and development. In addition, its genetic influence was low [15]. Overall, increasing genetic diversity of torbangun through gamma-ray irradiation was effective only at high dose level on plantlet height.

Conclusion

Results collected from the present study showed that the LD_{50} value of torbangun was 44,036 Gy. The combination both *in vitro* culture and gamma-ray irradiation can increase genetic diversity and phenotype response of torbangun, especially in low dose for leaf color and high dose for plantlet heigh. The various colors of leaf were produced in putative mutants with a dose level of 10 Gy and 20 Gy, it could be potential for torbangun as an ornamental plant which has a function also for health.

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Nutrient Management to Support *Blumea Balsamifera* (L.) DC Cultivation in Indonesia

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Abstract

Blumea balsamifera, one of medicinal plant from Indonesia, commonly used as raw material for *jamu* (Indonesian herbal medicine). The research was aimed to obtain proper nutrient management for *B. balsamifera* to produce standardized raw materials. *B. balsamifera* was cultivated at Manoko Research Installation (1,200 m asl), Lembang, Bandung, West Java, Indonesia from August to November 2014. Fertilizer dosages tested were (P1) control (without fertilizer), (P2) 20 t/h manure, (P3) 20 t/ha manure + 50 kg/ha urea + 50 kg/ha SP36 + 50 kg/ha KCl (20+50+50+50), (P4) 20+75+75+75, (P5) 20+100+100+100, (P6) 20+125+125+125, (P7) 20+150+150+150. The trial was arranged in randomized block design with four replications.

Manure, SP36 and KCl were applied at the time of planting, whereas urea was applied three times at time of planting, 1 and 2 months after planting (MAP). Plant samples were harvested at 3 MAP. Parameters observed were yield, quality and nutrient content and uptake. Fertilizer dosages affected yield significantly. The yield of *B. balsamifera* fresh leaves was the highest at 20 t/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl treatment (252 g/plant) and was significantly different from other treatments, while the lowest one was control (122.57 g/plant).

Saponin content of *B. balsamifera* ranged from 0.82 to 1.46% and tannin levels ranged from 3.66 to 4.50%. *B. balsamifera* simplisia quality of all treatments fulfilled IMM (Indonesian Medica Materia) standard except for ash content. Thus, fertilization dosage at 20 ton/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl was suitable for *B. balsamifera* cultivation to get high yield and produce standardized raw materials.

Keywords: Blumea balsamifera*, fertilizer, yield, quality*

Introduction

Blumea balsamifera is a medicinal plant that is widely used as raw materials for *jamu* (Indonesian herbal medicine). It grows wildly by the river and farmland up to 2,200 m asl. Roots and leaves are used traditionally in many ethnics in Indonesia such as in Bali and East Kalimantan.

The leaves of *B. balsamifera* extracted with 70% ethanol contained flavonoids, triterpenoids, quinones, tannins, and saponins which had antimalarial effect [1]. Tannin of *B. balsamifera* had antioxidant property [2] and was potential as antihyperlipidemic [3] along with flavonoid [4] and phenol [5], by fixing lipid profile. Meanwhile, saponin had antibacterial activity [6] and might involve in inhibiting heme polymerization as antimalarial although its mechanism was still unclear [1].

Quality of medicinal plants is closely associated with its active compound. It was significantly affected by genetic and environment factors [7]. Therefore, cultivation technology is important to support herbal medicine industries. Fertilization of both organic and inorganic fertilizer is important to amend soil fertility, promote plant growth and increase the content of secondary metabolite. The vegetative growth, yield, essential oil and crude oil of fennel plants (*Foeniculum vulgare* L.) were enhanced with the application of different mineral and organic fertilizers [8].

Another study on *Hyoscyamus muticus* plants indicated that the application of compost, organic manure (chicken manure) either alone or in combination with NPK, were positively affected on N, P, K content [9]. The yield with high active substance of *Plantago arenaria* also increased with the application of ½ (NPK at 100:100:50) with 300 ml/l tea compost [10]. Thus, mostly nutrient applications have positive effect on both yield and quality of medicinal plants. The objective of this study was to obtain suitable nutrient management for *B. balsamifera* to produce standardized raw materials.

Methodology

The field trial was conducted at Manoko Research Installation (1,200 m asl), Bandung, West Java, Indonesia from August to November 2014. The soil type in Manoko Research Installation was Andosol. The research was arranged in randomized block design, four replications. The plants were propagated using seeds. The seeds were sown in nursery bed for 2-4 weeks, and then transplanted into polybag filled with manure and soil (1:1). One-month old seedlings were then planted in the field. Plant spacing was 50 cm x 60 cm with plot size was 5 m x 3 m. Number of plants per plot was 50 plants. Factor tested were seven fertilizer dosages (P1) control (no fertilizer); (P2) 20 t/ha manure; (P3) 20 t/ha manure + 50 kg/ha urea + 50 kg/ha SP36 + 50 kg/ha KCl; (P4) 20 t/ha manure + 75 kg/ha urea + 75 kg/ha SP36 +75 kg/ha KCl; (P5) 20 t/ha manure + 100 kg/ha urea + 100 kg/ha SP36 + 100 kg/ha KCl; (P6) 20 t/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl and (P7) 20 t/ha manure + 150 kg/ha urea + 150 kg/ha SP36 + 150 kg/ha KCl.

Manure, SP36 and KCl were applied at the time of planting, whereas urea was applied three times (at time of planting, 1 and 2 months after planting (MAP). Plant samples were harvested at 3 MAP. The harvested plant samples were 5 plants/plot and the rest of the plants was harvested to be measured its total weight per plot. The harvest was performed by cutting the plants 5 cm from its base, then the stem was separated from the leaves. The fresh weight of stem and leaves was measured, and the biomass was dried in the oven at 50 °C to get dry weight of the stem and leaves.

Plant height parameter was measured every month. Other parameters included yield, nutrient content and uptake of leaf and stem, phytochemical content and proximate concentration was observed when the plants were harvested at 3 MAP. Soil and manure, simplicia quality (phytochemical content and proximate concentration), nutrient content and uptake were analysed at laboratory of Indonesian Spices and Medicinal Crops Research Institute. Data of plant height and yield were analysed statistically using ANOVA and further analysed using DMRT 5% if they were significantly different.

Results

Plant height and yield

Plant height at 3 MAP ranged from 33.28 to 42.00 cm. Fertilizer application gave no significant effect on plant height (Table 1). However, fertilization significantly affected leaf fresh and dry weight at 3 MAP (Table 1). The highest leaf fresh weight (252.40 g/plant) and dry weight (38.27 g/plant) was at 20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl treatment

(P6). Plant without fertilizer (P1) produced the lowest leaf fresh weight (122.57 g/plant) and dry weight (20.07 g/plant).

Nutrient content and nutrient uptake

The content of N and K in the stem was almost similar, whereas N content in the leaf was the highest compared to P and K. P content was the lowest both in stem and leaf. N stem content ranged from 1.88-2.22%, while in the leaf was 3.09-3.46%. The stem contained 0.37-0.38% P and in the leaf was 0.37-0.41%. As for K content, in the stem was 1.30-3.14% and in the leaf ranged from 1.56% to 1.88% (Fig. 1).

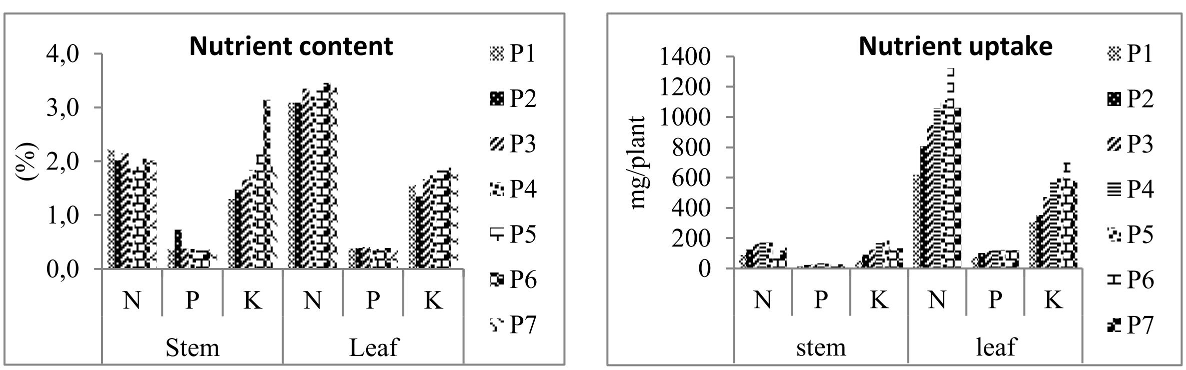
Nutrient uptake was the amount of nutrient absorbed by plants for its growth until harvested at 3 MAP (Fig. 1). As well as nutrient content parameter, the amount of N and P K in the stem was almost similar, but the amount of N transported to the leaf was the highest compared to K and P.

The amount of P was the lowest both in the stem and leaf. Although the data were not analysed statistically, there was tendency that nutrient uptake of plants in control treatment was lower than those with fertilization treatments.

Table 1. Plant height and yield of *Blumea balsamifera* at several fertilizer dosages at 3 months after planting

	Fertilizer dosage				**Plant height**	**Fresh weight of leaf**	**Dry weight of leaf**
	Manure (ton/ha)	**Urea (kg/ha)**	**SP 36 (kg/ha)**	**KCl (kg/ha)**	**cm**	**g/plant**	
P1	-	-	-	-	34.54	122.57 a	20.07 a
P2	20	-	-	-	42.00	166.24 b	26.10 ab
P3	20	50	50	50	36.44	183.11 bc	28.27 ab
P4	20	75	75	75	33.09	222.79 d	32.99 b
P5	20	100	100	100	36.56	216.04 d	33.09 b
P6	20	125	125	125	34.47	252.40 e	38.27 b
P7	20	150	150	150	33.28	196.14 bc	31.51 ab
CV						27.23	25.99

Means within any one column followed by the same letter were not different at DMRT 5%



P1	:	No fertilizer (control)
P2	:	20 kg/ha manure
P3	:	20 kg/ha manure + 50 kg/ha urea + 50 kg/ha SP36 + 50 kg/ha KCl
P4	:	20 kg/ha manure + 75 kg/ha urea + 75 kg/ha SP36 + 75 kg/ha KCl
P5	:	20 kg/ha manure + 100 kg/ha urea + 100 kg/ha SP36 + 100 kg/ha KCl
P6	:	20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl
P7	:	20 kg/ha manure + 150 kg/ha urea + 150 kg/ha SP36 + 150 kg/ha KCl

Fig. 1. Nutrient content and nutrient uptake (N, P, K) in the stem and leaf of *Blumea balsamifera* at 3 months after planting

Simplicia quality of Blumea balsamifera leaf

Based on proximate quality analysis, simplicia quality of *B. balsamifera* has fulfilled Indonesian Medica Materia (IMM) standard except for ash content which was slightly higher than requirement (Table 2). The active compound of *B. balsamifera* leaf measured in this study was saponin and tannins. Saponin content at 3 MAP was 0.82 to 1.46%, and tannin content was 3.66 to 4.50% (Table 2). Although data were not analysed statistically, plants with no fertilizer treatment tended having high saponin, whereas plants treated with medium dosage (P4 dan P5) contained higher tannin content.

Table 2. Simplicia quality of *Blumea balsamifera* leaf at 3 months after planting

Fertilizer treatment	Water content	Ash content	Acid-insoluble ash	Water-soluble extract	Alcohol-soluble extract	Saponin	Tannin
	%						
P1	8.73	10.68	0.64	21.14	8.56	1.46	4.02
P2	8.74	10.63	0.62	20.56	8.56	1.06	3.68
P3	8.77	11.40	0.81	20.53	9.39	0.87	3.66
P4	9.33	11.00	0.71	20.56	9.60	0.82	4.41
P5	9.31	10.24	0.64	19.43	9.24	0.87	4.50
P6	9.40	10.36	0.63	18.95	9.29	0.87	4.22
P7	9.55	10.68	0.65	19.64	9.66	1.00	3.75
IMM*	Max 11%	Max 10%	Max 4%	Min 11%	Min 5%		
Analysis method						TLC scanner	Spectrophoto metri

*IMM: Indonesian Medica Materia
P1 : No fertilizer (control)
P2 : 20 kg/ha manure
P3 : 20 kg/ha manure + 50 kg/ha urea + 50 kg/ha SP36 + 50 kg/ha KCl
P4 : 20 kg/ha manure + 75 kg/ha urea + 75 kg/ha SP36 + 75 kg/ha KCl
P5 : 20 kg/ha manure + 100 kg/ha urea + 100 kg/ha SP36 + 100 kg/ha KCl
P6 : 20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl
P7 : 20 kg/ha manure + 150 kg/ha urea + 150 kg/ha SP36 + 150 kg/ha KCl

Discussion

Mostly, the application of both organic and inorganic fertilizers indicated positive effect on yield of *B. balsamifera*. However, plant height was not affected significantly by fertilization. The same result was found on the plant height of *Thymus vulgaris* which was not significantly affected by fertilization [11].

The highest yield of fresh and dry weight of leaf indicated by the application of 20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl treatment (P6) (Table 1). This was due to promotive effect of nitrogen, phosphorus and potassium, which have been studied in many plants.

Nitrogen has important role in physiological process such as photosynthesis and carbohydrates synthesis [9], the build of amino acids, proteins, enzymes, and nucleic acids [12]. The nitrogen dosage from 50 to 150 kg N/ha enhanced basil herb yield 63.90% and gave considerable effect to phosphorus and potassium content [13]. However, the excessive nitrogen application could disturb plant metabolism leading even to poisoning. In this study, the dosage of 20 t/ha manure combined with 150 kg/ha of urea, SP36 and KCl (P7) indicated lower yield than P4, P5 and P6 treatments (Table 1). The decrease in basil herb yield also was reported following the increase of nitrogen dosage [13]. Sufficient N supply, besides increased production, also enhanced development and accumulation of primary and secondary metabolites [14], [15].

Based on soil analysis (data were not shown), the soil fertility was categorized as moderate fertility with low P, which was the common characteristic of Andosol. Phosphorus is important nutrient involved in the synthesis of nucleic acids, phospholipids and co-enzymes as well as a main constituent of energy compounds [16]. However, most phosphorus was unavailable in the soil, bounded by soil granules [17], hence limiting its availability. Phosphorus availability could be increased by manure application [18] and inorganic fertilization [19].

Potassium plays an important role in growth, yield and quality of crops. It usually occurred in high concentration mainly in the meristematic tissues and in the phloem [12]. The specific role of potassium in plant included protein synthesis, osmoregulation, photosynthate translocation, internal cation and anion balance and enzyme activation [20]. It also controlled the opening and closing of stomata which affected transpiration cooling and carbon dioxide uptake for photosynthesis [21]. Potassium deficiency could interrupt nitrogen metabolism caused the accumulation of harmful amino substances and ammonium ions in the plant (Nowacki 1980 in [12]).

The role of organic fertilizer such as cattle and sheep was mainly to enhancing soil aggregation, soil aeration, increasing water holding capacity and offers good environmental conditions for root system [22]. The N, P, K content of *Hyoscyamus muticus* plants were positively affected by the application of compost, organic manure either alone or in combination with NPK [9].

Fertilization also affected secondary metabolite content. Foliar fertilizer application contained N, P. Mg, K, Ca, Fe, Zn, Cu, Mo and B was reported enhancing twig numbers, tannin, saponin, Ca and P content of *Indigofera* spp [23], also increased saponin and tannin on *Ocimum gratissimum* [24]. On the other hand, study on *Panicum maximum*, indicated contrasted result.

Nitrogen application had no effect on saponin and tannin content [25]. However, inspite of fertilization, saponin content in this study was tended higher in control plant than treated plants (Table 2), might due to soil fertility which was low in P content. In previous study on *Quillaja saponaria*, the synthesis of saponins was higher in response to stress, indicated plant survival under adverse soil and climatic conditions [26]. Tannin content tended increasing with the application of 20 kg/ha manure + 100 kg/ha urea + 100 kg/ha SP36 + 100 kg/ha KCl, but decrease at higher dosages (Table 2). The same result was found on *Pinus sylvestris* which indicated lower tannin content at higher level of fertilizer [27]. Tannin was divided into two groups, condensed and hydrolysed tannin [28]. Tannin content might be affected or not by fertilization. Study on 35 woody plants revealed fertilizing significantly decreased concentrations of phenylpropanoids (including its derived compound, condensed tannins) but not of terpenoids or hydrolyzable tannins [29]. Tannin contained in *B. balsamifera* leaf might belong to condensed tannin group, since it was affected by fertilizer. However, this required further study.

Conclusion

The application of inorganic fertilizer in *B. balsamifera* cultivation was still required to get high yield. The dosage of 20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl gave the highest yield. Saponin content ranged from 0.82 to 1.46% and tannin content was 3.66 – 4.50%.

Simplicia quality in all treatments had fulfilled Indonesian Medica Materia (IMM) except for ash content. Nitrogen uptake was higher than P and K both in the leaf and stem of *B. balsamifera*.

The dosage of 20 kg/ha manure + 125 kg/ha urea + 125 kg/ha SP36 + 125 kg/ha KCl was suitable for *B. balsamifera* cultivation in Indonesia to get high yield and produce standardized raw materials.

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Climate Effect on True Seed of Shallots Production as Related to Number of Fertilizer and Benzyl-aminopurine Applications

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Abstrat

Climate and fertilizer play an important role in the true seeds of shallots (TSS) production while Benzylaminopurine (BAP) was reported to increase the size and number of the flower. The aim of this research was to determine the effect of climate as related to the number of fertilizer and BAP applications on TSS production. The experiments were conducted at Karo 3.1677° N, 98.5337° E and Toba Samosir Districts 2.30650N, 99.01750E (>1000 masl). The treatments were arranged in a split-split plot design with four replications wherein the location as the main plot, the number of fertilizer at 10, 6, and 3 times as subplot and BAP at planting time, 2, 3, 4, 5, and 6 weeks after transplanting (WAT) as sub-subplot. The BAP at planting time with concentration 37,5 ppm and at 3 WAT was increased number of leaf and seed, seed weight per plot, and 100 grains. Season, application time of fertilizer, and BAP have a significant influenced on growing plants, germination rate, and maximum growth potential. Solar radiation and environment is strongly influenced on seed formation and quality of seeds because related to low of disease and the high number of pollinators.

Keywords: Innovation, technology, true seeds of shallots, climate, fertilizer, and BAP

Introduction

Shallot (Allium cepa L. Aggregatum group) is a major source of vegetables and can affect economic inflation in Indonesia. The main problem in the farmer's level is the limitation of a good seed's quality. The alternative way is to overcome the shortage of planting material to increase the production and quality of shallots by developing seed planting material from seeds (generatively) known TSS.

The biggest obstacle of TSS production in tropical areas such as Indonesia is un-simultaneous flowering and seed least formed. The low percentage of flowering due to climatic conditions in Indonesia is unsuitable especially during off-season wherein the daily is 50-100 mm day^{-1}. High rainfall and humidity affected on plants susceptible to diseases such as antraknos, alternaria, and powdery mildew which results in broken stems at the stage of seeds formation, unformed seeds, and unfilled out. The ideal growth of shallots is a low temperature around 7-12 °C with long photoperiodities ranging >12 hours for the initiation of flowering [1] and for increasing the size of flowers (umbels) and accelerating blooms interest 12-18 °C with altitude around 1000 m above sea level [2]. Furthermore, there are indications those for the formation of capsules and seeds, the weather conditions in the lowlands are more suitable than the highlands especially in off-season.

Another factor that is important in stimulating flowering to produce a high quality of seeds is fertilization and application of growth regulators. The growth and yield of shallots can increase with the application of nitrogen, phosphate and potassium fertilizers. The average needs of N, P, K, and manure for TSS production were 254.5 kg ha^{-1} [3], 126.5 kg ha^{-1} [4], 96 kg ha^{-1}, and 10 t ha^{-1} chicken manure [5]. To increase TSS production, flowering induction is by adding cytokinin growth regulator such as BAP. The application of BAP 20-50 ppm produces the highest production in Chamomile plants [6], 50 ppm increases the flowering percentage to 156% and a concentration of 150 ppm produces 8.0 bulbs per clump [7]. The results of this activity are expected to answer the problem in the future in producing TSS in appropriate climate, fertilizer, and BAP application according to plant growth stage.

Materials and Methods

Experimental site

The experiments were conducted on upland areas in two districts are Toba Samosir and Karo, North Sumatra during the wet season (WS) and the dry season (DS). The two fields were Gurgur (latitude: 02°18'37" N, longitude: 99°1'11" E, 1223 m a.s.l.) and Tongkoh (latitude: 03°11'40" N, longitude: 98°32'24", 1340 m a.s.l.).

Weather parameters

Cumulative precipitation (mm), solar radiation (MJ m^{-2}), humidity, and mean minimum temperature (°C) were collected from Gabe Parent Seeds Center and Tongkoh experimental station. Daily climate data is used to determine the positive and negative factors in TSS production in two different climates.

Experimental design

The treatments were arranged in a split-split plot design wherein the location was the main plot, the number of fertilization applications as subplots, and Bensylaminopurine as sub-sub plots. The variety used was Bima Brebes stock seeds class in WS and the same seeds after harvest in DS with a dormancy and vernalitation period of 1 month, respectively. Planting is carried out on September 22, 2016 (WS) and February 28, 2017 (DS). As the main plots were L1: Tongkoh (wet climate, 1340 masl) and L2: Gurgur (dry climate, 1223 masl), sub plots the number of NPK fertilizer applications at three treatments, N1: 3 times, N2: 6 times, N3: 10 times, and sub-sub plots were gibereline G1: during planting, G2: 2, G3: 3, G4: 4, G5: 5, and G6: 6 WAT to obtain 18 treatment combinations in each study location. The experiment was 1.2x2 m (2.4 m^2) with six treatments each sub plots.

Land preparation and crop management

Soil fulled tillage, beds were made with width = 1.2 m, height = 30-40 cm, distance between beds = 50 cm, and length adjusted with conditions facing the East-West. Before mulch was installed with a spacing of 0.2 x 0.2 m, the application of chicken manure was 10 t ha^{-1} and Furadan then stirred with soil. Seed vernalization was carried out at 10 ^{0}C for 4 weeks then immersion with Benzylaminopurine (BAP) with a concentration of 37.5 ppm and drained up to dried and mixed with Dithane M-45 fungicide dose of 2 g kg^{-1} seed. Planting shallots in the planting hole that has been made and selected with medium-sized and healthy bulb seeds then watering. Two weeks after planting or before flowering plants shaded with bamboo with white plastic transparent as a roof. The application of NPK fertilizer with a dose of 600 kg ha^{-1} where N1 was 3 times applications (10 DAP, 42 DAP, and 75 DAP), N2 was 6 times applications at 10 DAP and follow every 11 days until the age of 75 DAP, N3 was 10 times at 10 DAP and continue every week until the age of 75 DAP by being cast on the soil. BAP with a dose of 37.5 ppm, it was applied at the time of planting and other treatments were added by 37.5 according to the treatment were G1 (control), G2 (2 WAP), G3 (3 WAP), G4 (4 WAP), G5 (5 WAP), and G6 (6 WAP) by spray to the plant. Pest and disease control using yellow traps and selective pesticide until seed filling.

Watering and removing of dew, weeding, removing old leaves, and installing raffia strings on the rows of plants were other treatments to enforce flower stalks. Planting targets to attract pollinators is done before planting and the application of pollinator insects when the flowers bloom. First harvesting at the age of 110 HST and carried out + 5-6 harvests with intervals of 4-7 days.

Plant and soil (sampling and measurement)

The measurement of vegetative and generative growth was at 20, 30, 40, 50 days after planting (DAP) and harvest. Measurement on 0.16 m^2 (2 rows x 0.4 m) and on 0.64 m^2 (4 rows x 0.8 m) for harvest area wherein was not surrounded by missing hills. Plant growth was included plant height, number of splits, and leaves while the yield components were the amount of TSS/umbel, TSS/umbel weight (g), TSS weight/plant (g), weight 100 grains (g). Soil samples for initial soil analysis were taken before land preparation and carried out for pH, organic C, total N, P and K-HCl, P-Bray parameters, exchange cations (Na, Ca, Mg, and K), KB, and texture analyses.

Statistical Method

Data obtained from location and treatment was subjected to analysis of variance (ANOVA).

Data coefficient of variation (CV) was visually inspected by plotting residuals to confirm homogeneity of variance before statistical analysis. The comparison of time means was made by Tukeys's Honest Significant Difference (HSD) test (0.05) using Statistical Tool for Agricultural Research (STAR) software. Microsoft Excel© was used for determining the descriptive statistics for each of the measured soil and plant variables.

Results and Discussions

Soil Factors

The results of soil analysis such as pH, organic carbon, total nitrogen, phosphorus and potassium-HCl, exchange bases, texture, cation exchange capacity (CEC), and base saturation (BS). The data showed that the soil pH in the both locations was acidic category (Tongkoh = 5.97 and Gurgur 5.89). C-organic status in both locations were high (5.06%) and medium (2.92%) while the total N of the two locations was medium (0.50% and 0.34%), respectively. Based on the land history, areas were potato plantations which used a high organic fertilizer ± 10 t ha^{-1} which served to maintain fertility and soil moisture. According to [2], shallots grow and well develop if planted at an optimum altitude of 0-1000 masl, sufficient organic matter, and neutral pH. Potential P and K content were very high for both locations (2192.31 mg 100 g^{-1} and 1623.02 mg 100 g^{-1} for P_2O_5 and 91.03 mg 100 g^{-1} and 168.16 mg 100 g^{-1} for K_2O, respectively). Inversely of P available at Gurgur with very low status (0.85 ppm) and medium in Tongkoh (9.39 ppm). Phospor is an important element for shallots in terms of root extension and leaf development, bulb size, production, the bulb ripening process, and increasing the strength of flower stems [8, 9]. Based on the [10], to obtain shallot production of 6.6 t ha^{-1} at peat soil in Malaysia estimated that P uptake is 5 kg ha^{-1}, while the proper application of P to be quickly absorbed and available to plants is placing close to the bulbs [8, 11].

Both locations contained very high sand >77% (sandy clay texture). This type of texture is favourable in shallots production because have crumb soil structure, medium to clay texture, and good drainage and aerase [6]. Exchange rates for both locations differ in terms of K-dd where Gurgur has a very high exchange rate (1.21 me 100 g^{-1}) and medium at Tongkoh (0.44 me 100g^{-1}). Likewise, Ca-dd was the high category at Gurgur (10.63 me 100 g^{-1}) and medium at Tongkoh (7.53 me 100g^{-1}). Otherwise, Na-dd and Mg-dd content was moderate status for both locations where each of them is 0.54 me 100g^{-1} and 1.48 me 100g^{-1} at Tongkoh 0.35 me 100g^{-1} and 1.21 me 100g^{-1} at Gurgur. The percentage of CEC depends on the percentage of exchange bases, the higher the base availability, the greater the availability in soil solutions which can ultimately increase soil pH [12]. Besides functioning to increase soil pH, exchange bases such as Calcium and Magnesium are also important nutrients for the growth of shallots. The CEC is high category in both locations (38.88 me 100g^{-1} and 28.12 me 100g^{-1}) while BS is low at Tongkoh (25.69%) and medium at Gurgur (47.33%). Both locations were horticultural areas (potatoes) which used large amounts of organic fertilizer which is an important factor contributing to high CEC in sandy soils. This is supported by [12], sandy soil has a low CEC compared to loamy and silty clay soils where it is necessary to add organic matter because plays a role in holding and exchanging cations/bases. On the other hand, BS in both locations is categorized as low to moderate, this is related to the pH is rather acidic, because some of the absorption complex is occupied by Al^{3+} and H^{+} cations. Based on the soil data that both of locations suitable for shallots production.

Climate Factors

Climate data during plant growth for both period with the number of days for all phases of plant growth of 133 days. The lowest number of days was 18 days in the early flowering period until the capsule is formed 10% and the highest was 62 days in the 10% capsule until harvest in WS.

Based on the number of days in a particular growth stages, the lowest rainfall in the planting to the umbel appears at 3 and 18 mm respectively in Gurgur and Tongkoh (average 0.15 and 0.9 mm per day) where requires watering actions to minimize stress. On the other hand, the mean daily rainfall on three phases was >5 mm. Rainfall started at 37 and 35 DAP (19 and 26 mm) at each station and was highest at 40 and 43 HST (39 and 44 mm). There was a slightly decreased in the initial phase of flowering until the capsule formed 10% with a total rainfall of 167 and 96 mm (daily average 9.3 and 5.1 mm) and increased in the 10% capsule phase until harvest, especially in Tongkoh of 478 mm (7.7 mm). Shallot crop can produce pithy bulbs when water in a field capacity until bulb formation [13]. Furthermore, at a depth of 25 cm water table is best water availability conditions for growing the number and weight of bulbs per hectare [14]. The highest mean wind speed in the planting to the umbel appears stage (199.8 km h^{-1}) at Tongkoh. Similarly, humidity, the highest mean in Tongkoh experimental station for all growth stage. Based on the results of [11], the prover relative humidity for shallots growth and development is 50-70%.

Humidity >70% at the study site has an impact on the increased in the attacked of anthracnose fungi in all locations at Tongkoh and 80% at Gurgur.

The DS 2017 showed the sufficient rainfall at the beginning of growth (>7 mm per day) and decreased in bulb stage appears to the beginning of the flower blooms, to 10% capsules, and harvest (5.8 mm, 4.7 mm, and 3.7 mm, respectively). Generally, at the beginning of the growth until the harvest of all climate parameters is higher in DS 2017 compared to WS 2016/2017, except the humidity has decreased starting the bulb appears to the beginning of the flower blooms until the harvest. This was very beneficial for plants because the number of pollinators was increased and supported by the surrounding environmental conditions where there are many coffee plants that bloom throughout the year. Based on [15], the percentage of flowering will increase if the average temperature is <8 °C. This is appropriate with the results in the field that 98% of the plants produced flower and 60% have begun to form capsules while anthracnose attacked around 80% that resulted the stem broken and the capsules formed unmatured. Solar radiation was higher at early stages at Gurgur in WS compared to DS. Solar radiation has increased since the age of 13 DAP and was higher than WS in Gurgur and in Tongkoh. Solar radiation has a positive impact on photosynthesis and shallots production. Photosynthesis increase was required by a sink, because number and quality of yield relied on an adequate source and sink relationship [16]. Furthermore, photosynthesis had positively related to leaf area index (LAI) and yield [17].

Vegetative Growth

The results showed that the NPK fertilizer and BAP had an effect on plant growth of shallot.

There is a significant difference between season and fertilization applications on plant height at 40 DAP (Table 1). The interaction of fertilizer application and the BAP showed significantly difference on plant height at 20 DAP in WS and leaf number at 50 DAP at two seasons in Gurgur (Table 2 and 3). Application of 50 kg N ha^{-1} and 375 kg P_2O_5 ha^{-1} in several splits is economically sound to produce the highest bulb yield of shallot [18]. According to [19], growth regulators such as cytokinin are non-nutrient organic compounds that play a role in supporting and changing plant physiology like cell division and enlargement. Giving BAP can increase plant shoot formation and play an active role in increasing plant height, length and breadth of leaves [20]. Table 2 showed significant difference on interaction the fertilizer applications and BAP on plant height at 20 DAP in WS. Growth regulating like BAP are small amounts of active organic compounds that give biochemical, physiological and morphological responses to plants. On the other hand, application of N, P and K fertilizers to support plant growth and production[2], nitrogen used to leaf growth and development, improve the green colour of leaves, and the formation of shoots/tillering [21] phosphate is a crucial part of macromolecule as phospholipids and nucleic acids that can influence the number and quality of yield [18], while potash is a crucial on energy status of the plant, translocation, storage of assimilates and maintenance of tissue water relation, and crop quality.

Furthermore, plant height at DS showed a significant increase compared to WS (Table 1).

Altitude and climate affected plant height, leaf width, and biomass while planting time affected flowering of shallots [22]. Increase in solar radiation during dry season partly contributed to yield increase and as a driving factor of potential grain yield [23]. The interaction between BAP and fertilizer applications showed a significant effect on leaf number at 50 DAP in the treatment of N1B2 and N1B6 in Table 6. The best BAP application time after soaking was 3 WAP with the application of fertilizer as much as 10 times not significantly difference from 6 times on leaf number at 50 DAP.

Table 1. Effect of fertilizer application time and season on plant height at 40 DAP and flower number per plant at 30 DAP at Gurgur (period Sept 2016 and Feb 2017).

Treatment (T)	Plant height at 40 DAP (cm)			Flower number per plant at 30 DAP		
	N1	N2	N3	N1	N2	N3
MH 2016	36.0 bA	33.2 bA	35.4 bA	1.5 aA	1.1 bB	1.2 bB
MK 2017	41.1 aB	46.7 aA	45.6 aA	1.6 aA	1.6 aA	1.4 aB

Within columns, means followed by the same lower-case letter are not significantly different according to HSD Test 0.05.

Within rows for a given parameter, means followed by the same upper case letter are not significantly different according to HSD Test 0.05.

Yield Components

The yield components included number of splits and flowers, TSS weight per plant, also 100 grains weight in Table 2, 3, and 4. The interaction of BAP and location showed significant differences on number of splits at 50 DAP in WS 2016/2017 (Table 2). In both locations, the applications of BAP at 3 weeks after planting had a positive effect on number of splits at 50 DAP.

Table 2. Effect of fertilizer and BAP application on plant height and number of tillers at Tongkoh and Gurgur experimental station period Sept 2016 to Feb 2017.

T	Plant height at 20 DAP (cm)			Number of splits at 50 DAP		Flower number per plant at 40 DAP					
						Location 1			Location 2		
	N1	N2	N3	L1	L2	N1	N2	N3	N1	N2	N3
B1	15.0 bB	17.7 Aa	16.3 aA	6.9 abA	5.8 abA	3.2 aA	3.8 aA	3.2abA	2.1 aA	1.3 aA	2.0 aA
B2	15.8 bB	16.5Aab	17.1 aA	6.7 abA	6.1 abA	3.3 aA	3.8 aA	2.4 bB	2.3 aA	1.3 aB	1.7 aAB
B3	18.9 aA	15.3 aA	18.6 aA	6.2 abA	6.8 aA	3.5 aA	4.0 aA	4.4 aA	1.9 aA	1.8 aA	2.2 aA
B4	17.2abA	16.3 Aa	16.6 aA	5.7 bA	6.0 abA	3.9 aA	3.1 abA	3.6abA	1.9 aA	2.2 aA	1.4 aA
B5	16.7abA	16.8 Aa	16.9 aA	6.4 abA	5.5 bA	3.4 aB	4.3 aA	2.8 bB	2.0 aA	1.7 aA	2.1 aA
B6	15.7 bA	16.2 Aa	16.6 aA	7.0 aA	6.2 abA	3.3 aA	2.2 bB	3.5abA	2.1 aA	1.9 aA	1.9 aA

Within columns, means followed by the same lower-case letter are not significantly different according to HSD Test 0.05.
Within rows for a given parameter, means followed by the same upper-case letter are not significantly different according to HSD Test 0.05.

Table 3. Effect of BAP and fertilizer application time on leaf number at 50 DAP and flower number per plant at 40 and 50 DAP at Gurgur experimental station for two seasons (period Sept 2016 and Feb 2017).

T	Leaf number at 50 DAP			Flower number per plant at 40 DAP						Flower number at 50 DAP		
				Season 1			Season 2					
	N1	N2	N3	N1	N2	N3	N1	N2	N3	N1	N2	N3
B1	26.8aA	22.8aA	25.9aA	2.0aA	1.3aA	2.1aA	3.0aA	2.2aB	2.2bB	3.6aA	3.0aA	3.3abA
B2	22.1aB	28.3aA	28.1aA	1.7aAB	1.3aB	2.3aA	2.0aB	2.7aAB	2.8abA	3.2aA	3.3aA	3.4abA
B3	24.1aA	27.0aA	28.5aA	2.2aA	1.9aA	2.1aA	2.2aB	3.0aA	3.5aA	3.1aB	3.2aB	4.0 aA
B4	24.1aA	25.7aA	27.6aA	1.4aB	2.2aA	1.9aAB	2.3aA	2.5aA	2.7abA	3.1aA	3.7aA	3.4abA
B5	26.8aA	25.2aA	23.8aA	2.1aA	1.7aA	2.0aA	2.5aA	2.5aA	2.3abA	3.4aA	3.2aA	2.9 bA
B6	22.9aB	25.7aAB	27.5aA	1.9aA	1.8aA	1.9aA	3.2aA	2.5aAB	2.3abB	3.9aA	3.0aB	3.7abAB

Within columns, means followed by the same lower-case letter are insignificantly different according to HSD Test 0.05.
Within rows for a given parameter, means followed by the same upper-case letter are not significantly different according to HSD Test 0.05.

The highest number of splits was in B3 treatment which gave a significantly difference ($P<0.001$) with treatment B5 and B6. Likewise, inter-season showed a significant decreased ($P=0.04$) DS 2017 compared to WS 2016/2017 [24], the cytokinin hormone in BAP besides influencing metabolic processes can also stimulate the growth of shoots and cells. From the results of this study indicate that the application of the second BAP at 3 WAP increased the number of splits by 13.8-17.2% compared to at 5 and 6 WAT [5], the BAP applications independently influences the number of seeds and flower per umbel, also the percentage of flowering plants.

Table 3 showed an interaction between BAP and fertilizer of each location on flower number per plant at 40 and 50 DAP. Planting time and fertilization affect flower production. According to [25], the main factors of flower production are photoperiod, temperature and water availability.

The flower number per plant was significantly affected by BAP and fertilizer application on several treatments. Low concentrations of BAP can increase the number of flowers per bulb and the flowering plants percentage, and a concentration of 50 ppm can encourage flowering of shallots in the highlands [5]. In all fertilization treatments, the highest flower numbers at DS.

Table 4. Effect of fertilizer application time and BAP on TSS weight per plant and 100 weight grains at Gurgur experimental station at two seasons (period Sept 2016 and Feb 2017).

Treatment	TSS weight per plant (g)						100 grains weight (g)
	Season 1			Season 2			
	N1	N2	N3	N1	N2	N3	
B1	0.271 bA	0.209 abA	0.191 cA	0.413 aA	0.443 Aa	0.468 aA	0.361 abA
B2	0.266 bA	0.256 abA	0.316 abA	0.397 aA	0.457 Aa	0.426 aA	0.363 abA
B3	0.394 aA	0.263 abB	0.386 aA	0.437 aA	0.454 aA	0.392 aA	0.369 aA
B4	0.290 abAB	0.317 aA	0.210 bcB	0.421 aA	0.431 Aa	0.424 aA	0.363 abA
B5	0.296 abA	0.195 bB	0.289 abcA	0.423 aA	0.433 Aa	0.470 aA	0.359 bA
B6	0.229 bA	0.256 abA	0.312 abA	0.452 Aa	0.431 aA	0.386 aA	0.360 bA

Within columns, means followed by the same lower-case letter are not significantly different according to HSD Test 0.05.
Within rows for a given parameter, means followed by the same upper-case letter are not significantly different according to HSD Test 0.05.

The NPK application 6-10 times with the cast system greatly helps nutrient availability according to the plant growth phase, where nitrogen plays a role in the formation of amino acids, nucleic acids, and nucleoproteins, phosphorus functions in root development and increases yield [8], and potassium play an important role in plant metabolism, and regulate turgor cells [26]. The timing and technique of fertilizer application can affect the yield of shallots[27]. The availability of nutrients during the process of plant growth by providing inorganic and organic fertilizers produced a high quality of seeds. Likewise, the high amounts of N can increase Persian bulbs production by 18 and 13% [28] and almost 42% fresh bulbs [29]. Table 4 showed the interaction of BAP and fertilizer on TSS weight per plant while BAP application affected 100 grains weight.

The application of N2 was significantly difference in WS. The BAP and fertilizing interactions showed insignificant effect on TSS weight per plant in DS. Increase in solar radiation during dry season partly contributed to yield increase and as a driving factor of potential grain yield [23].

Seeds quality

The germination rate and maximum growth potential varied with BAP, fertilizer application, and interaction. The highest germination rate and maximum growth potential was N3B4 (88.7%) and N2B3 (90,3%), respectively. The Interaction was a significant difference n N1B5 (81.4%) on germination rate and N1B2 (80.2%) and N3B1 (81.9%) on maximum growth potential.

Application BAP at 3 WAP and fertilizer application 6 times increased the germination rate and maximum growth potential. Based on [30], application eight times of fertilizers with a dose of 600 kg NPK ha^{-1} produced higher seed per hill while [31] effect of synthesized α amylase and potassium nitrate which is important in terms of seed germination speed.

Conclusions

Climate and altitude affect onion flower production, but in the seed, formation is strongly influenced by climate. Dry climate with average humidity <80% and solar radiation mean >50% produces an average production of >400 gr. Low disease and the high number of pollinators in the dry season to give success and production of TSS. Similarly, the application time of fertilizer and BAP have a significantly influenced on vegetative, generative, germination rate, and maximum growth potential. BAP at planting time with concentration 37.5 ppm and at 3 WAT and 6-10 times fertilizer application is the best to increase TSS production.

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Postharvest Characteristics of Torch Ginger (*Etlingera elatior*) Inflorescence at Two Developmental Stages

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Abstract

Torch ginger (*Etlingera elatior*), which flourishes in tropical and subtropical climates, with extravagant and showy inflorescence can be used as cut flower at each developmental stage. The objective of this study was to elucidate the postharvest performance of the torch ginger inflorescence treated with different vase solutions. Inflorescences at tight bud (TB) and torch shows (TS) stage at about 35 and 58 days respectively, after emerging from rhizome were used.

Sucrose (20 g/L), 8-hydroxyquinoline sulphate (8-HQS, 0.1 g/L) and distilled water were used as vase solution treatments. Postharvest characteristics, including vase life, relative fresh weight (RFW), dry weight, solution uptake rate, respiration and ethylene production rate were evaluated.

The results showed that vase life of TB inflorescence was significantly higher than TS irrespective of treatment used. Contrary, significant higher solution uptake rate was shown in the TS inflorescence. In dry weight and respiration rate measured, there was no significant difference between the two inflorescence developmental stages. No ethylene was detected in all treated inflorescences throughout the study. Prolongation of vase life was associated with an increase in RFW. An increase in RFW was found in TB inflorescence but reduction in RFW was shown in TS inflorescence at the end of vase life. Supplementation of sucrose promoted the opening of true flowers. The use of 8-HQS and distilled water enable the development of true flowers, but they failed to open. Results revealed that sucrose treated inflorescences both in TB and TS showed better postharvest quality compared to 8-HQS and distilled water.

Introduction

Torch ginger (*Etlingera elatior* (Jack) R.M. Smith) inflorescence with striking colour and showy appearance is an important trait as a cut flower. Depending on the market demand, the inflorescences from the tight bud to the full bloom stage could be used as a cut flower [1].

However, limited studies were conducted to reveal the postharvest performance of this leafless inflorescence especially at advanced developmental stages. For cut flower usage, the supplementation of sugars and biocides are commonly used as postharvest treatment to extend the vase life of the flower. Hence, the objective of the current study was to evaluate the postharvest performance of torch ginger inflorescence at two developmental stages treated with different vase solutions. In this study, sucrose and 8-hydroxyquinoline sulphate (8-HQS) were used as vase solution treatment. Understanding the postharvest physiology and characteristics of torch ginger inflorescence is important in order to develop it as a cut flower. This would be an added advantage for the development of proper postharvest handling in future.

Materials and Methods

Torch ginger inflorescences at two developmental stages i.e. tight bud (closed and pigmented bud) and torch shows (all outer involucre bracts unfolded) stage, at about 35 and 58 days respectively, after emerged from rhizome were harvested from Farm 2, Faculty of Agriculture, University Putra Malaysia, Malaysia (3°00’21.34’’N, 101°42’15.06’’E, 37 m elevation). The harvested inflorescences were transported to Laboratory of Postharvest, Faculty of Agriculture and prepared as described by Choon and Ding [2]. Sucrose (20 g/L), 8-hydroxyquinoline sulphate (8-HQS, 0.1 g/L) and distilled water were used as vase solution. Inflorescences were kept at 22 °C, 55-65% relative humidity and 5.50 µmol/m^2/s of light intensity (12 h/d).

The inflorescences were observed daily for vase life assessment. The vase life of inflorescence was considered ended when 50% of the bract area wilted. Vase solution update, relative fresh weight, respiration and ethylene production rate were determined every two days. At the end of vase life, inflorescence was collected. The dry weight of the inflorescence was measured after over drying. The experiment was conducted in a randomized complete block design. Five replicate inflorescences were used for postharvest performance determination. The experiment was repeated two times. Data were analysed using ANOVA (SAS Version 9.2, 2010) and means comparison were performed using Duncan’s multiple range tests (DMRT).

Results

Vase life

From the observation, the vase life of torch ginger inflorescence was limited by the browning on bracts surface. Results indicated that there was significant interaction between inflorescence developmental stages and vase solution treatments on vase life of torch ginger inflorescences (table 1). At tight bud stage, vase life of inflorescences was significantly extended by 23.2% when sucrose solution and distilled water were used compared to 8-HQS (Fig. 1). At torch shows stage, no significant difference of vase life was found in all the treated inflorescences (Fig. 1).

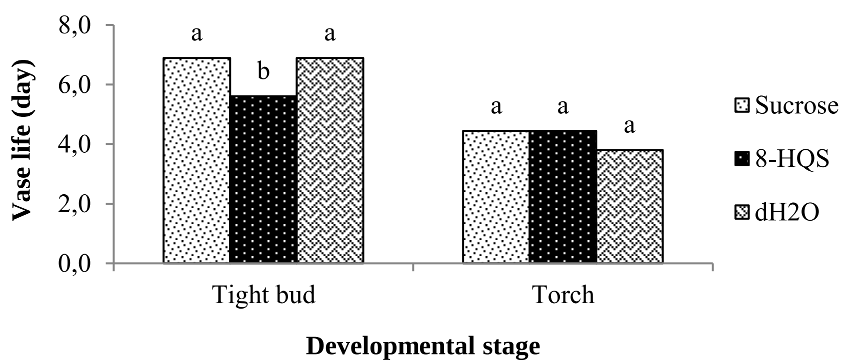

Fig. 1. Vase life of inflorescences at two developmental stages treated with sucrose, 8-hydroxyquinoline sulphate (8-HQS) and distilled water (dH_2O). Means of different vase solution within the same developmental stage followed by the different letters are significantly different by DMRT at P≤0.05.

Table 1. Main and interaction effects of sucrose, 8-hydroxyquinoline sulphate (8-HQS) and distilled water on vase life, solution uptake, relative fresh weight, dry weight and respiration rate on tight bud and torch shows stage inflorescences.

	Vase life (day)	Solution uptake (ml/2 d)	Relative fresh weight (%)	Dry weight (%)	Respiration rate (ml CO_2/kg/h)	Ethylene (nl C_2H_4/kg/h)
Stage (S)						
Tight bud	6.4 a[z]	12.0 b	1.6 a	7.7 a	34.1 a	n.d.
Torch shows	4.2 b	27.1 a	-1.5 b	7.8 a	34.5 a	n.d.
Treatment (T)						
Sucrose (20 g/L)	5.7 a	19.0 a	1.6 a	8.1 a	34.0 a	n.d.
8-HQS (0.1 g/L)	5.0 b	17.9 a	-1.3 c	7.2 b	34.8 a	n.d.
Distilled water	5.3 ab	16.9 a	-0.2 b	7.8 ab	34.0 a	n.d.
S x T	**	*	**	ns	ns	n.a.

[z] *For each treatment, means within a column followed by the same letter are not significantly different by DMRT at $P \leq 0.05$.*
ns,,** Non significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.*
n.d.=not detectable.
n.a.=not applicable.

Vase solution uptake

There was significant interaction between inflorescence developmental stages and vase solution treatments on solution uptake rate (table 1). At tight bud stage, inclusion of sucrose and 8-HQS significantly improved the vase solution uptake of inflorescences by 52.1 and 31.9%, respectively, as compared to distilled water (Fig. 2). However, no significant difference of vase solution uptake in torch shows stage inflorescences (Fig. 2).

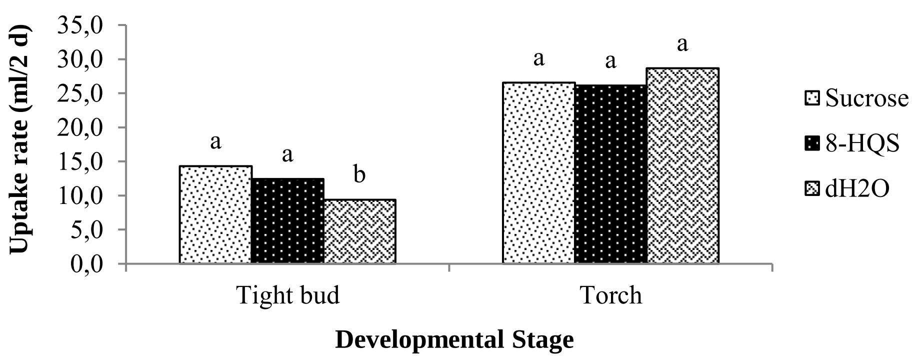

Fig. 2. Solution uptake rate of inflorescences at two developmental stages treated with sucrose, 8-hydroxyquinoline sulphate (8-HQS) and distilled water (dH_2O). Means of different vase solution within the same developmental stage followed by the different letters are significantly different by DMRT at $P \leq 0.05$.

Relative Fresh Weight (RFW) and Dry Weight

There was significant interaction between inflorescence developmental stages and vase solution treatments on RFW (Table 1), Tight bud stage inflorescences that treated with sucrose and distilled water showed a positive gain of RFW at the end of vase life as compared to day 0 (Fig. 3). In 8-

HQS treated inflorescences, reduction of RFW was found. A decrease of RFW was shown in torch shows stage inflorescences at the end of vase life. There was no significant difference in dry weight in the inflorescences being used in this study (Table 1). Inflorescences treated with sucrose showed significant higher dry weight than 8-HQS but had no significant difference with distilled water (Table 1).

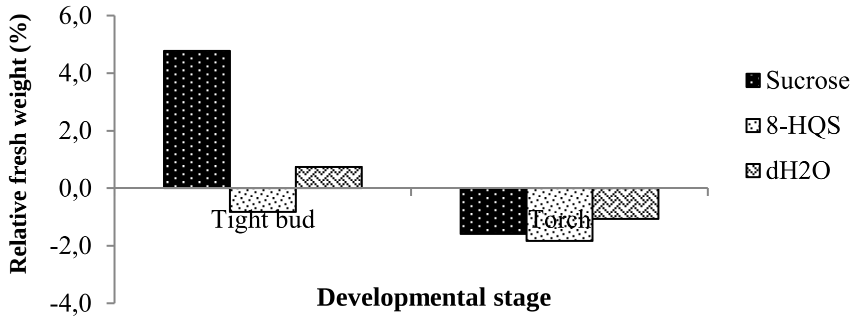

Fig. 3. Relative fresh weight of inflorescences at two developmental stages treated with sucrose, 8-hydroxyquinoline sulphate (8-HQS) and (dH_2O). Means of different vase solution within the same developmental stage followed by the different letters are significantly different by DMRT at $P \leq 0.05$.

Respiration and ethylene production

There was no significant difference in respiration rate measured in inflorescence of both developmental stages and vase solutions used (Table 1). No ethylene was detected in all treated inflorescences throughout the study (Table 1).

Discussion

The results revealed that the use of sucrose and distilled water as vase solution effectively extended the vase life of inflorescence at tight bud stage as compared to 8-HQS. At torch shows stage, the vase life of inflorescence was not affected by the vase solution applied. From the observation, inclusion of sucrose in the vase solution enabled the opening of bracts of tight bud stage inflorescence as vase life progressed. However, no bracts opening was found in inflorescences treated with 8-HQS and distilled water. Besides, bract browning symptom was chronic in inflorescence treated with 8-HQS and distilled water as compared to inflorescences treated with sucrose solution. The use of sucrose also promotes the opening of true flowers of inflorescence at torch shows stage. The true flowers of inflorescence treated with sucrose solution developed and opened on day 1 after harvest and continued as vase life progressed. At the end of vase life, at least three rings of true flowers were developed and opened. The true flowers of torch shows stage inflorescence treated with 8-HQS and distilled water were developed but they failed to open at the end of vase life.

In torch ginger inflorescence, the use of 8-HQS neither extends vase life nor promotes bracts or true flowers opening indicating that there is inadequate supply of sugars to the inflorescences.

Besides, the bracts browning symptom was delayed in inflorescences treated with sucrose as compared to 8-HQS and distilled water. Similar results were found in *Sandersonia* sp., *Iris* sp., and carnations that exogenous sugar solution delays visible senescence in petals [3]. Therefore, sucrose is important in vase life prolongation and promotes the opening of bracts and true flowers in cut torch ginger inflorescences. From the results, the changes of RFW in inflorescences seem associates with its vase life. The positive gain of RFW in inflorescences at tight bud stage treated with sucrose and distilled water had longer vase life. A decrease of RFW caused shorter vase life as shown in inflorescences at tight bud stage treated with 8-HQS and all inflorescences at torch shows stage.

Conclusion

The results showed that the two developmental stages of cut torch ginger inflorescences responded differently to the solution treatments. Sucrose could be used as postharvest vase solution as it promotes the opening of bracts at tight bud stage and true flowers at torch shows stage of the torch ginger inflorescences.

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MODERN AND URBAN FARMING SYSTEM FOR HEALTHY AND SAFETY VEGETABLE PRODUCTS

Heterosis of Yield Character and Fruit Fly (*Bactrocera Dorsalis*) Resistance on Chili

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Abstract

Fruit fly oviposition on chili fruit may cause considerable damage. Heavy attack of this pest will decrease both yield quantity and quality, and will affect the crop economic value. Using resistant variety is one of the ways to suppress fruit damage. This study was aimed at determining the heterosis value of the yield and fruit fly resistance. The research used Randomized Block Design that consisted of 15 treatments (9 F1 generations, 3 resistant parents and 3 susceptible parents) with three replications. A total of 990 chili plants was used in this study. Plant morphology was evaluated for plant performance (plant height, stem diameter, leave length, leave width, flowering date, fruit length, fruit width, fruit weight, yield per hectare) and fruit fly resistance. The results showed that the F1 generation had a better high yielding trait compare to the parents. For fruit fly resistance, F1 generations were more resistance than the susceptible parents. Index deterrent oviposition F1 generations were higher than susceptible parents. The heterosis phenomenon obtained in both characters, yield and fruit fly resistant.

Keywords: oviposition, hybrid, F1 generation

Introduction

The lack of chilies stock on the rainy season is the main factor that significantly increases the selling price resulting in the raising on the inflation rate. Aryasita *et al.*, [1] research showed that the yield of chilies from relatively same fields in the rainy season was not as much as in the dry season. In the rainy season, the price of chili greatly increases to more than IDR 100.000. for every kilogram, while it stays below IDR 10.000. in the dry season [2]. The price fluctuations cause chili to become the highest contributor to inflation [3].

Fruit fly is a pest for chilies in rainy and dry seasons. In the rainy season, the attack of the fruit flies can cause the loss of crops up to 100% [4], [5], [6], [7]. The population of the fruit flies significantly increases in rainy season comparing to dry season [8], [9], [10]. The fruit fly species that mostly attack Indonesian chili cultivation confirmed as *Bactrocera Dorsalis* [11], [12].

One of the strategies to control fruit fly pests is using the resistant varieties. The combination of resistant variety and high yield variety into one variety is the expected breeding target. This combination can be reached using heterosis phenomenon, where the heterosis is a condition where the phenotypic generation offspring is better than the parents [13]. The crossing of fruit fly resistant variety with high yield variety produced several combinations offspring, but the heterosis information from the offspring is unknown [14]. The research aimed to obtain the information on heterosis value of F1 generation from the crossing of high yielding chili and fruit fly resistant chili.

The result of the research can be a consideration for choosing the best crossing combination for creating high yield and fruit fly resistant chili variety.

Materials and Methods

Heterosis testing was carried out in the Indonesian Vegetable Research Institute (IVEGRI) research station in the rainy season (May to December 2017). The experiments using Randomized Block Design (RBD) with three replicated. The treatment was 15 varieties, consisted of 9 F1 generation, 3 susceptible parents and 3 resistant parents. The experiment plot was $6m^2$ and consisted 24 plants/plot with 80 cm x 50 cm planting distance. The technically cultures using IVEGRI standard.

The observation was plant height/PH (cm), plant width/PW (cm), leaf width/LW (cm), leaf length/LL (cm), date of flowering/DF (days), fruit length/FL (cm), fruit width/FW (cm), fruit weight/FWG (g), yield (t/ha). The resistance test using choice method [15]. The parameter of resistance was oviposition deterrent index [16], [17], [18].

Analysis of morphological and yield character carried out using analysis of variance (ANOVA) and continued using test Scott Knots test. Analysis of resistance to infestation of fruit fly's data using Wasse's criteria [19]. Prediction heterosis values of each character were calculated over mid-parent [20], [21], [22].

Results and Discussion

F1 generations, results from crossing between resistant and susceptible to fruit fly infestations, had characteristics that were not statistically different from the two parents for the character of plant height, stem diameter, leaf length, age to flower and fruit length, but showed significant differences for leaf width characters , fruit width, weight per fruit, and yield per hectare. Previous research showed that the morphological character of chili which is correlating positively with the fruit fly infestations level was fruit size [18]. The excellence of F1 generations can be seen from the yields per hectare which the yields of F1 generations showing a better value comparing to the parents (Table 1).

Table 1. The morphological and yield characters of F1 generations and the parents

Treatments	PH (cm)	PW (cm)	LW (cm)	LL (cm)	DF (days)	FL (cm)	FW (cm)	FWG (g)	Yield (t/ha)
F1 (4x1)	58.0 a	0.9 a	9.2 a	3.5 ab	53.0 a	10.4 a	1.1 c	6.4 b	21.2 ab
F1 (4x2)	64.5 a	1.0 a	9.4 a	3.4 ab	56.0 a	10.0 a	1.1 c	5.5 b	18.2ab
F1 (4x3)	65.0 a	1.0 a	10.6 a	3.7 ab	53.0 a	11.1 a	1.2 c	6.5 b	21.7 ab
F1 (5x1)	62.0 a	0.9 a	8.8 a	3.9 ab	54.0 a	10.4 a	1.0 c	4.6 b	15.2 ab
F1 (5x2)	62.0 a	1.0 a	9.8 a	3,9 ab	55.0 a	10.2 a	1.1 c	5.3 b	17.7 ab
F1 (5x3)	62.0 a	0.9 a	9.3 a	3.7 ab	53.0 a	11,6 a	1.1 c	6.5 b	21.5 ab
F1 (6x1)	64.0 a	1.1 a	6.7 a	2.5 b	51.0 a	11.9 a	1.1 c	5.7 b	19.0 ab
F1 (6x2)	65.0 a	1.0 a	9.5 a	3.6 ab	54.0 a	12.9 a	1.2 c	7.4 b	22.3 a
F1 (6x3)	63.5 a	1.0 a	9.1 a	3.4 ab	53.0 a	12.0 a	1.5 b	8.1 a	22.1 ab
Parent 1	44.0 a	0.8 a	8.0 a	3.1 b	65.0 a	10.1 a	0.7 d	2.4 b	12.8bc
Parent 2	57.0 a	0.8 a	10.6 a	3.9 ab	62.0 a	8.2 a	0.7 d	1.9 b	9.9c
Parent 3	57.5 a	0.8 a	9.0 a	3.0 b	55.0 a	10.6 a	0.8 d	3.2 b	17.1 ab
Parent 4	49.0 a	1.0 a	10.3 a	3.8 ab	53.0 a	8.9 a	1.6 b	8.5 a	11.3 bc
Parent 5	50.0 a	0.8 a	7.5 a	3.8 ab	57.0 a	10.8 a	1.4 b	9.6 a	12.8bc
Parent 6	55.5 a	1.0 a	8.8 a	3.7 ab	51.0 a	11.8 a	1.8 a	12.2 a	16.3 ab
CV (%)	13.7	10.4	11,7	9.7	3.4	17,5	9.0	24.5	22,0

Abbreviations: PH=plant height; PW=plant width; LW=leaf width; LL=leaf length, DF=Date of flowering, FL=fruit length, FW=fruit width (cm), FWG=Fruit weight.

The choice method was used for the assessment of chili resistance to fruit flies. In this method, the fruit flies were given the chance to choose all treatment as an oviposition place [18]. The research result showed that F1 generations oviposition deterrent index and parents were so diverse.

They started from 27.3% to 100%. Oviposition deterrent index of 100% meaning that fruit flies were not oviposited the chili. Therefore, the chili had the criteria of highly resistant. The smaller index value of oviposition deterrents meaning the chili more susceptible the chili to fruit fly infestations. The F1 generation had a higher oviposition deterrent index value compared to susceptible parents, but was still lower than the resistant parent (Table 2).

Table 2. Resistance level of F1 generations and the parents

Treatments	Oviposition deterrent index (%)	Resistance Criteria (Wasse *et al*., 2012)
F1.1 (4x1)	64.3	MR
F1.2 (4x2)	70.7	MR
F1.3 (4x3)	74.2	MR
F1.4 (5x1)	70.7	MR
F1.5 (5x2)	67.1	MR
F1.6 (5x3)	75.5	R
F1.7 (6x1)	67.1	MR
F1.8 (6x2)	70.7	MR
F1.9 (6x3)	70.7	MR
Parent 1	100.0	HR
Parent 2	100.0	HR
Parent 3	100.0	HR
Parent 4	33.0	MS
Parent 5	27.3	MS
Parent 6	30.0	MS

Abbreviations: R=resistant; MR=Moderately Resistant; HR=Highly Resistant; MS=Moderately susceptible

The traits transfer from resistant parents to susceptible that is having a high nutrient power expected to get a combination of high yielding properties and fruit fly resistance into one new superior variety. The F1 generation had a better high yielding trait comparee to one of its parents.

F1 had yield/hectar 18.5%-99.7% higher than its parents (Table 3). For resistance character, F1 was more resistance than the susceptible parents. Index deterrent oviposition F1 was higher than susceptible parents (Table 4).

Table 3. The heterosis of F1 yield character (t/ha)

Treatments	Susceptible Parents	Resistant Parents	F1 (offsprings)	Heterosis (%)
F1.1 (4x1)	11.3	12.8	21.2	75.93
F1.2 (4x2)	11.3	9.9	18.2	71.70
F1.3 (4x3)	11.3	17.1	21.7	52.82
F1.4 (5x1)	12.8	12.8	15.2	18.75
F1.5 (5x2)	12.8	9.9	17.7	55.95
F1.6 (5x3)	12.8	17.1	21.5	43.81
F1.7 (6x1)	16.3	12.8	19.0	30.58
F1.8 (6x2)	16.3	9.9	22.3	70.23
F1.9 (6x3)	16.3	17.1	22.1	32.34

Table 4. The heterosis of F1 resistance level-Oviposition deterrent index (%)

Treatments	Susceptible Parents	Resistant Parents	F1 (offsprings)	Heterosis (%)
F1.1 (4x1)	33.0	100.0	64.3	-3.31
F1.2 (4x2)	33.0	100.0	70.7	6.32
F1.3 (4x3)	33.0	100.0	74.2	11.58
F1.4 (5x1)	27.3	100.0	70.7	11.08
F1.5 (5x2)	27.3	100.0	67.1	5.42
F1.6 (5x3)	27.3	100.0	75.5	18.62
F1.7 (6x1)	30.0	100.0	67.1	3.32
F1.8 (6x2)	30.0	100.0	70.7	8.77
F1.9 (6x3)	30.0	100.0	70.7	8.77

Conclusion

F1 generations had a better high yielding trait compare to the parents. For fruit fly resistance, F1 generations were more resistance than the susceptible parents. Index deterrent oviposition F1 generations were higher than susceptible parents. The heterosis phenomenon obtained in both characters, yield and fruit fly resistance.

Acknowlegment

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Sustainability in Horticulture Production – Opportunity and Challenge for Innovative Technologies in Asia and Europe

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Abstract

Innovative technologies for production of horticultural commodities should be sustainable, that means they should follow the three pillars philosophy – economic growth, environmental protection and social progress. Very important is a 'Land Suitability Analysis and Evaluation' in order to find out the appropriate land for the different horticultural crops. Very often, farming systems are very traditional, but all commodities, fruits, ornamentals and vegetables should be produced with efficient, sustainable and innovative technologies. Technologies are necessary to improve the production safety also under unfavourable cultivation conditions in all steps of crop production. Suitable method for plant propagation is important, in case of generative methods to secure high germination rate or vegetative in this case preferable using tissue culture. Use of plant growth promoters are often helpful in particular under stress conditions as high temperature, high salt concentration in the soil or drought. Regarding plant protection, some biological control methods will be presented with use of humic acid and plant oils. One possibility is using protected cultivation systems. Sometimes the soil conditions are not suitable for intensive and sustainable crop production use of soilless cultivation methods are recommended as Substrate Culture with trickle irrigation, Aeroponic and the newer system is the Aquaponics. Finally, will be highlighted in some examples the interaction between growing conditions, in particular the light condition, and the internal compounds of vegetables, in this regard the nutritive value and marketable quality.

Keywords: Land use evaluation, Plant propagation, Bioregulators, Biological control, Soilless culture, Marketable quality

1. Introduction

With the growth of the population, in particular in countries as Indonesia, it can be observed a significant growth of a demand on horticultural crops in particular fruits and vegetables. Therefore, should be improved the intensity of the land use and their productivity under consideration of the sustainability. There are many approaches to define "Sustainability". Often, are highlighted three pillars – economic growth, environmental protection and social progress – (Adams, 2006). Very often, these three aims are in contradiction, on one hand the land should be used with high profit, on the other hand aspects of the environment and social conditions should not be neglected (Sumner, 2008). Poincelot (2004) pointed out that sustainable horticulture is not just an adopted practice, but also a system that includes the individual farms, as well the local ecosystems, and the communities. In order to cultivate horticultural crops sustainable from high importance are land suitability analyses in the regions, considering the influences of physical, social, economic, infrastructure and environmental issues (Huynh and Böhme, 2005). Another point related to sustainable vegetable production is the diversification this includes also the use of indigenous crops well adapted to the growing conditions. Use and conservation of the plant biodiversity existing in the South East Asian regions can be a guaranty for sustainable food supply and is important in the fight against malnutrition.

Very often, farming or cultivation systems are very traditional, but all commodities, fruits, ornamentals and vegetables should be produced with efficient, sustainable and innovative technologies. Technologies are necessary to improve the productivity and the production safety under unfavourable cultivation conditions in all steps of crop production Böhme *et al*., 2010. Some innovative cultivation methods and technologies related to cultivation of horticultural crops investigated and developed in our lab will be presented.

2. Evaluation of Horticultural Land Use Systems

In order to investigate the level of interactions between the criteria in the ‘three pillars’ it is necessary to use methods which are able to evaluate different land use strategies in agriculture and horticulture.

Therefore, a sustainable land use strategy needs a multicriterial evaluation of the current situation in order to develop and compare different horticultural land use Scenarios. In former investigations multicriterial evaluation method were used in order to compare and evaluate different cultivation systems in horticulture (Böhme, 1986). In order to evaluate different land use systems, the “Analytic Hierarchy Process” (AHP) following Saaty (1994) was used as a more convenient method for evaluation of complex issues also using the criteria selection and weighting.

In frame of the AHP were investigated – 15 sub-criteria, based on three main-criteria related to economy, ecology and socials aspects (Table 1).

All these sub-criteria were discussed with the stakeholders of the province and the land users of the communities (Fig. 1).

Furthermore, were agreed five scenarios for the land use in the investigated communities:

CFS1 – Tree nursery, Pig husbandry, Aquaculture

CFS2 – Vegetable growing, Pig husbandry, Aquaculture

CFS3 – Rice growing, Pig husbandry, Aquaculture

CFS4 – Rice growing, Poultry production, Pig husbandry

CFS5 – Vegetable growing, Poultry production, Pig husbandry

The Inputs into the AHP were based on data analysis and observations later on, following the criteria land users at the community evaluated and weighted in workshops the criteria and sub-criteria. Later on, the evaluation by the farmers was used in the further calculation. Based on this evaluation and weighting, was calculated a ranking of the sustainability of the five scenarios for agricultural and horticultural land use in the investigated communities.

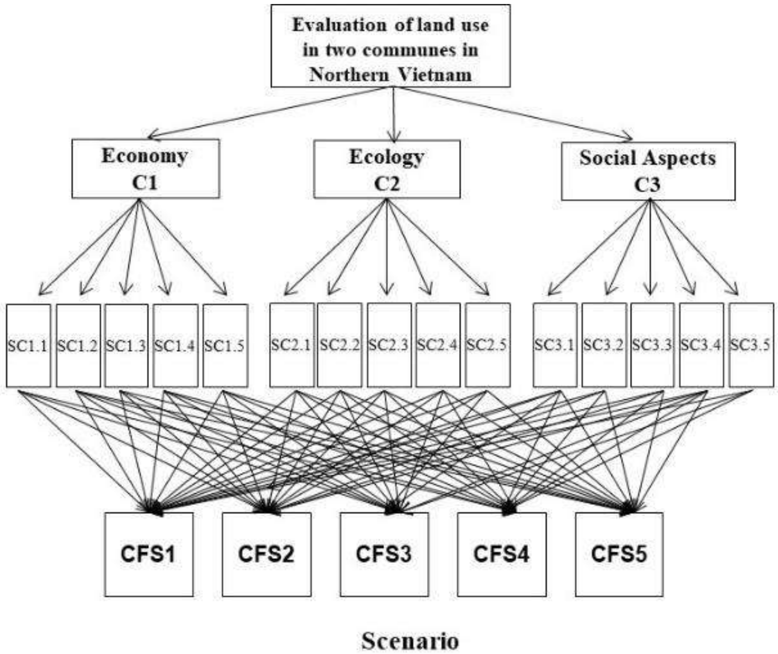

Fig. 1. Methodology for multicriterial evaluation, definition of main and sub-criteria for evaluation of five scenarios CFS1…, CFS5 (Dao, 2016)

Table1. Final Result of Weighting and Ranking of five Farming Systems by the farmers by the farmers (land users)

Sub-Criteria	**Weighting factors for the five Farming System Scenarios**					
	Wi	CFS1	CFS2	CFS3	CFS4	CFS5
E1. Infrastructure in the Province and Communes	0,0254	0,0114	0,1196	0,0436	0,0152	0,0026
E2. Area of land per household	0,0761	0,0430	0,1121	0,0153	0,0045	0,0059
E3. Marketing conditions	0,0939	0,0394	0,1757	0,0310	0,0041	0,0018
E4. Crop yield and productive capacity of livestock and fishes	0,1328	0,0709	0,1213	0,0294	0,0060	0,0029
E5. Relation Revenue: Expenditure	0,3051	0,1654	0,1280	0,0238	0,0061	0,0037
Eco1. Use of Pesticides and fertilizers	0,1090	0,0033	0,0023	0,0141	0,0384	0,1037
Eco2. Soil quality and fertility	0,0309	0,0008	0,0018	0,0093	0,0449	0,1414
Eco3. Irrigation systems	0,0363	0,0186	0,1348	0,0355	0,0067	0,0020
Eco4. Influence on the Biodiversity	0,0363	0,0009	0,0015	0,0076	0,0421	0,1525
Eco5. Waste management	0,0479	0,0246	0,1321	0,0219	0,0092	0,0038
SA1. Job opportunities	0,0048	0,0001	0,0012	0,0101	0,0541	0,1299
SA2. Working conditions	0,0108	0,0057	0,1119	0,0296	0,0082	0,0036
SA3. Education and qualification	0,0188	0,0006	0,0043	0,0143	0,0322	0,1294
SA4. Motivation of the land users	0,0253	0,0104	0,1692	0,0392	0,0039	0,0017
SA5. Social services	0,0465	0,0245	0,1013	0,0127	0,0116	0,0069
Total hierarchy Ni	1,0000	0,4196	1,3172	0,3375	0,2871	0,6919
Ranking		**3**	**1**	**4**	**5**	**2**

The first place in the ranking (Table 1) that means the most promising Farming system is the combination of – Vegetable growing, Pig husbandry and Aquaculture –. In general, it can be emphasized following advantages:

- From economical point of view following the experience of the farmers, this land use system has the highest efficiency.

- From ecological point of view, this land use system the advantages are the recycling of organic waste as food for pigs or fishes, as organic fertilizer or for biogas production.
- Considering the criteria including social aspects can be highlighted the high work load and therefore good job opportunities and the possibilities for balancing of the workload during the year.

Following this ranking, most promising for the farmers seems to be land use systems including the horticultural farming systems with vegetable cultivation or forest nursery. The results of the AHP analysis are not mandatory for the farmers, but this is a way to make clear, beside economic criteria, also environment and social aspects are necessary to consider. The positive outcome of the discussion in the workshops and the evaluation of the criteria is a much better understanding of the way to organise the land use sustainable.

3. Methods for Plant Propagation

There are several reasons not to propagate plants, in particular vegetables, generative by using seeds or vegetative by using cuttings. Sometimes a low germination rate can occur or it is necessary to have plant material free from viruses or other diseases. In such cases, in-vitro methods are a better propagation method, also very useful for breeding.
In this regard will be described two examples for using in-vitro methods breeding of water spinach (*Ipomoea aquatica* L.) and propagation of potato (*Solanum tuberosum* L.) in order to receive minitubers as seed potatoes.

Micropropagation of Water Spinach

The four steps of water spinach micropropagation are demonstrated in Fig. 2. Sterilized seeds were germinated in vitro. Germination rate of seeds was very low. After 2 weeks, only up to 30% of seeds had been germinated. Westphal (1994) reported that germination rates of water spinach seeds are usually low (<60%).

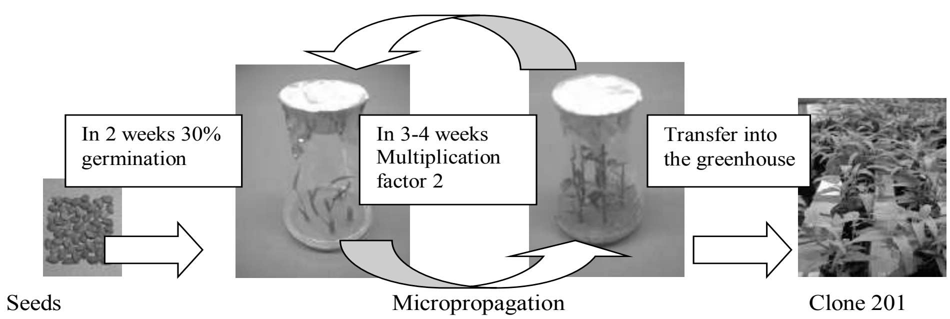

Fig. 2. Origin and propagation of clones used for these experiments

From the germinated seeds, the most vigorous ones were selected and 4 weeks after sowing nodal segments were cut. For propagation of clones, shoots have been subculture every three to four weeks. After four subcultures 18 clones with 12 to 24 shoots per clone were available. For acclimatization and transfer into the greenhouse, those clones with best multiplication rate and plant quality have been selected. Results for greenhouse evaluation of seven clones are presented here.

Greenhouse Cultivation

Greenhouse experiments have been conducted in three periods: 1) October until February, 2) April until September, 3) April until October. Results of the last period will be presented, as shoot number and fresh weight per plant of the investigated clones (Fig. 3). Because water spinach will be sold in bundles of 20 to 30 cm long shoots two parameters were considered to characterize the marketable yield: the number of shoots formed per plant and the fresh weight harvested. Both parameters differed in dependence of the genotype.

Four weeks after transplanting into the greenhouse plants started to from 2 to 8 shoots per plant.

Five clones had in average four to five shoots. Clone 205, however, had only about three shoots and clone 209 formed in average six shoots per plant.

In addition, concerning the fresh weight that was harvested after two weeks cultivation clone 205 had the lowest yield with about 25 g per plant and clone 209 had the highest yield with 52 g fresh weight per plant. The other clones had between 30 to 40 g fresh weights per plant, however, these differences were not significant. Plant density was 34 plants per m^2 that means the yield per m2 ranged from 7.6 kg for clone 205 and 16 kg for clone 209 in four months harvesting period.

Westphal (1994) reported yields per year up to 400 t per ha under upland cultivation. That means with clone number 209 this yield could be expected even under greenhouse condition in temperate climate.

Larkcom (1991) suggested harvest intervals from 3 to 4 weeks. In this experiment, harvest intervals were shorter. The results showed that in hydroponics two-week-intervals are possible.

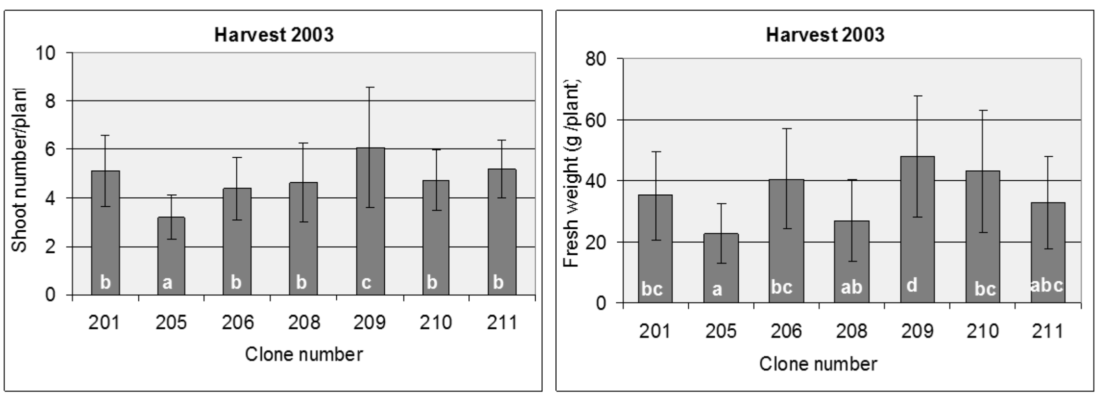

Fig. 3. Influence of genotype on the yield (shoot number and fresh weight per plant). Means from nine harvests in two-week-intervals from 28.05. until 30.09

Botanical Characteristics of Clones

Clones could be characterized by parameters like leaf number, shoot length and vine formation.

Mean leaf number ranged in dependence on the genotype from six to eight leaves per shoot.

Clones number 205 and 208 had the lowest leaf number with about six leaves per shoot. Clones 206 and 209 had the highest ones with about eight leaves per shoot. Nevertheless, clone number 209 had very short shoots. Clones number 205 and 209 formed short and uniform shoots. Vine formation was very scarce in these clones. Shoots of 20 to 30 cm are harvested for use. For intensive cultivation, shoots of uniform length are preferred, therefore, clone 209 seems to be the most suitable one for production purposes. Leaf shape of the clones investigated was stable and a characteristic feature. Clones number 205 and 209 were long and narrow-leaved ones. Clones number 201, 208, 210, and 211 had rather short and broad leaves, heart-shapes ones.

Micropropagation and Minituber Production of Potato in Aeroponic System

The plantlets (Solanum tuberosum L. cv. Marfona) were obtained by successive in vitro subcultures on modified MS (Murashige and Skoog, 1962) called culture medium supplemented with 0.5 mg l-1 thiamine, 100 mg l-1 myo-inositol, 0.5 mg l-1 pyridoxine, 0.5 mg l-1 biotin, 30 g l-1 sucrose and was solidified with 7 g l-1 agar (Serva Kobe I). The plantlets were removed from the culture medium after two weeks and they were transplanted directly under non-sterile conditions in small pots (Ø 4cm) filled with perlite in order to induce root formation. The plantlets were acclimatized for four days under a humid environment, at photon flux density of 60 µmol m-2 s-1 and a temperature of 23-25 °C, where they developed roots easily. After four days, they were transferred to the greenhouse for three weeks to produce small potato plants of 8 to 10 cm in length with 4 to 5 single leaflets.

Aeroponic System

Aeroponic cultivation system consisted of an upper and a lower part, a tank to collect the nutrient solution, a pump and spraying units with control panel and timer. The lower part was divided in eighteen closed sections of a 60 x 72 x 35 cm covered by Styrodur® (BASF, Germany) sheets. Acclimatized plants were transplanted on 4th April in small rockwool cubes and then fixed in holes (Ø 4 cm) in the Styrodur sheets. About 2/3 of the potato shoot was placed into the lower part. Potato shoots grew in the upper part while roots, stolon's, and tubers developed in the lower part under dark conditions. The nutrient solution was periodically sprayed using four fog nozzles per closed sections (every 5 min for 20 sec). The nutrient solution compositions were N, P, K, Ca, Mg and Fe at the concentrations of 180, 50, 300, 200, 60 and 3 mg l-1 in the first month for shoot and stolon development and then 60, 50, 300, 200, 60 and 3 mg l-1 during minituber growth, respectively. The nutrient solution was renewed every two weeks. In the nutrient solution, the pH (5.5 to 6.5) and EC (2 to 2.5 mS cm-1) were daily controlled and adjusted. The plants were stabilized in the upper part in dependence of the shoot length using four layers of wire meshes.

The plant growth regarding to leaf number (48-56 leaves) and shoot length (174-181 cm) per plant was not strongly affected by the plant density. However, the leaf area was larger at lower plant densities. When the supply of nitrogen was decreased 30 days after transplanting, the plants reduced the growth and increased tuber induction. Tuber formation started after three weeks and 100% of the plants induced minitubers after seven weeks of transplanting (Fig. 4). After 120 days, leaves of cv. Marfona became senescent. Thus, our aeroponic plants finished their life cycle approximately four months after transplanting which was one month longer than in soil or substrate culture in greenhouse for an early cultivar like Marfona. Increased vegetative growth and delayed tuberization was observed when the N supply was increased in the nutrient solution of aeroponically-grown potato plants (Ritter *et al*., 2001).

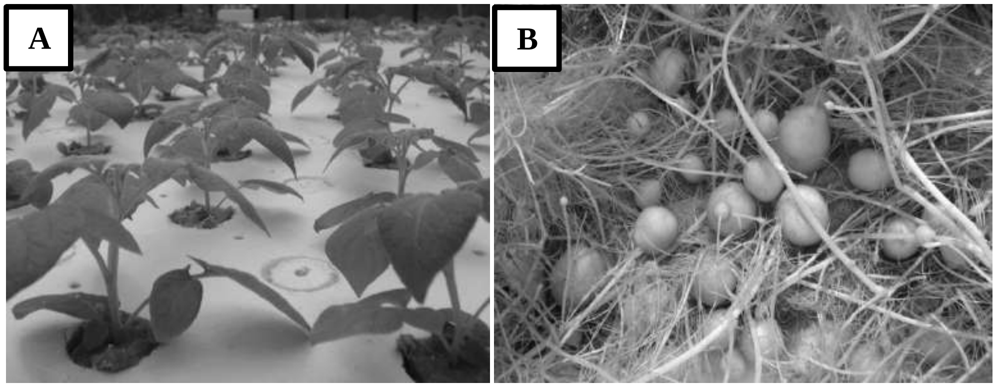

Fig. 4. (A, B). Potato plant development and minituber production using aeroponic system
A: Acclimatized plants one week after transplanting in aeroponic.
B: Growth and development of two months old potato plants.

Effect of Plant Density on Minituber Yield

The minituber harvesting began two months after transplanting into aeroponic system and lasted for two months. Though tubers continued to develop on older stolon's, most of them were formed on secondary new stolon. Significant differences in minituber number and weight per plant were found for all plant densities, but not per m^2 (Table 2). The average minituber weight ranged in between 10 and 11.8 g, probably due to the harvest criteria and the harvesting intervals used (Table 2) but was significantly affected by plant density.

Table 2. Minituber production (per plant and per m2) and tuber characteristics obtained using the aeroponic system.

Plants per m^2	Tuber weight kg m^{-2}	Tuber weight g $plant^{-1}$	Tuber weight (g/tuber)	Tuber >20 mm per plant		Tuber >20 mm per m^2	
				number	Weight (g)	number	Weight (kg)
25	12.0 *	479.5 a	11.8 a	32.2 a	446 a	804.3 *	11.1 *
35	11.9 *	341.5 b	10.8 ab	23.8 b	320 b	832.9 *	11.2 *
50	11.3 *	226.1 c	10.0 b	16.3 c	208 c	813.7 *	10.4 *

*Different letters in one row represent significant differences (Tukey P=0.05). * Not significant*

At the first harvest, the low plant density (25 plants/m^2) produced the highest yield in average of 60 g and 3.9 tubers per plant, which is two to three times the yield of that from 35 and 50 plants/m^2. The tuber number obtained in our aeroponic system was within the expected range of about 1130 minitubers per m^2 (Ritter *et al*., 2001). The highest total minituber yield per m2 was obtained at 25 plants per m^2 (12 kg per m2).

Regarding to minituber production per plant, the plant density had a significant effect. The tuber production per plant was significantly higher at 25 plants/m^2 with 40.82 tubers per plant and 479.5 g tubers per plant (Table 2). The highest minituber number in total and regarding tubers larger than 20 mm per plant was obtained with 25 plants/m^2.

4. Plant Growth Promoters

Biostimulators on growth of vegetables in hydroponic system

Biostimulators could be used to stabilize hydroponically systems and to counteract stress situations. Plant cultivation in hydroponic systems is quite problematic as to the proper balancing of EC and pH values. The application of bioregulators was tested as an approach to improving both the nutrient balance and plant growth. According to previous investigations, humates, lactates and *Bacillus subtilis* seem to be particularly suitable in this respect.

There are many good reasons for applying humic acid to encounter suboptimal growth conditions in the root zone or on the leaves. Humic acid improves the nitrogen uptake of plants and enhances the transport and availability of micronutrients in the plant (Tattini *et al*., 1990).

Humic acid effects turned out to be more pronounced when one or several growth factors (temperature, humidity, light, water and nutrient amounts, salinity, etc.) are above or below optimal levels for a short time (Hoang, 2003).

Organic substances sometimes enter into a rather stable bond with metal ions, particularly in the form of chelates. Lactates (lactic acid salts) turned out to be a stable form of the chelates.

Lactates entering into stable bonds with different metal ions are now available under the brand name LACTOFOL® (Shaaban *et al*., 1995). That suspension fertilizer was designed mainly for foliar application and patented as a plant growth and development regulator. Hardly any information has been available so far about its effects when applied to the root system.

Beside the organic substances, an organic preparation – *Bacillus amyloliquefaciens* (former B. subtilis) – was tested for its stress-reducing effects. At beginning of the experiments, the strain Bacillus subtilis FZB 24 of the Abitep GmbH-Berlin was used, isolated from soil, multiplied and formulated as a preparation. However, Bacillus subtilis may also produce beneficial effects on plant growth and resistance, for example, by breaking down available nutrients (Böhme *et al*., 2016). Tomato inflorescences were more readily fertilized after treatment with Bacillus subtilis (Abou Shaar, 1988). That agent was also found to increase the root and shoot fresh matter of tomato plants and stimulate the formation of adventitious roots. In former experiments, the effect of a combination of these three components were very helpful in stress situation during plant growth, in particular in case of suboptimal EC or pH values (Böhme, 1999). In these experiments this bioregulators was used as supplement to the nutrient solution. Lactates are used as leaf bioregulators and fertilizer, but it was not investigated whether humates and B. subtilis can have similar effects by leaf application.

The experiment aimed to investigate the effect of lactic acid (LACTOFOL O), K-Humate and *Bacillus subtilis* as biostimulators in substrate culture of *Cucumis sativus* cv. Jessica. All substances were applied separately or in combination on leaves and roots respectively, in total eight different treatments have been compared with the control. For each treatment 20 ml of 0.08% LACTOFOL O, 0.2% K-Humate or 107 cfu/ml spore suspension of Bacillus subtilis were applied three times in weekly intervals during the 5 till 10 leaf stage.

A three times application of biostimulators in the growing stage (week 4, 5, and 6) affected growth and yield of cucumber plants. The application of all substances tested stimulated the shoot development represented by a higher fresh matter of shoots and leaves in most variants (Figure 5).

Obviously, the location of application was important for the effect of the biostimulators. The application in the root zone led in each case to a higher fresh matter compared to the control. If the substances were applied over the leaves, the effect on shoot fresh matter was not as strong as if they were applied in the root zone. The application of Bacillus subtilis even resulted in a lower shoot fresh matter. The effect on leaf fresh matter was also a stimulating one. It should be stressed, however, if the combination of all substances was applied the effect was opposite stimulating if applied over the roots and inhibiting if applied over the leaves.

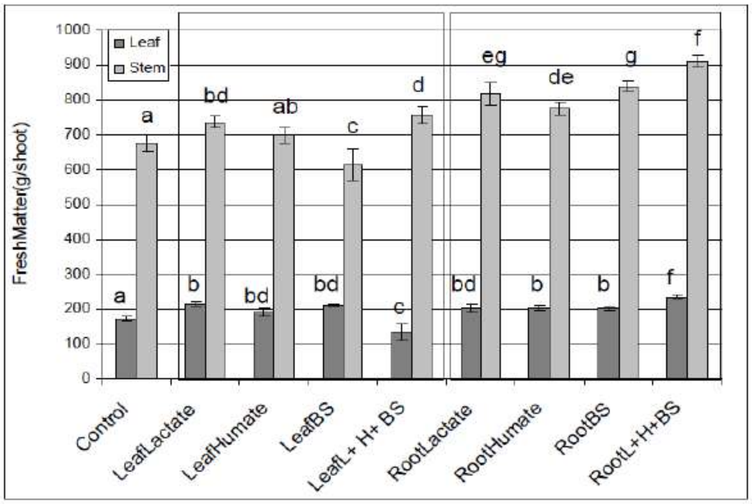

Fig. 5. Effect of application biostimulators (Lactate, K-Humate, Bacillus subtilis) on leaves and roots respectively on biomass of shoots and leaves after finishing the experiment
Different letters indicate significant differences (LSD, P=0.05)

Fruit Harvest and Quality

The total yield after one month harvesting (Figure 6) was affected after the application of biostimulators. The fresh weight of all cucumbers with market quality was about 500g; therefore, the number of fruits is representative for yield. The number of marketable fruits was higher than in the control in most variants treated with biostimulators. Especially the treatment with biostimulators on the roots resulted in a higher yield in the first harvest. Based on this the number of fruits finally harvested was considerably higher after treatment of roots with LACTOFOL and *Bacillus subtilis*. For the following experiments, further application also during the fruit set should be taken into consideration because these additional applications could enhance the yield further.

This could be especially important in long time cultivation.

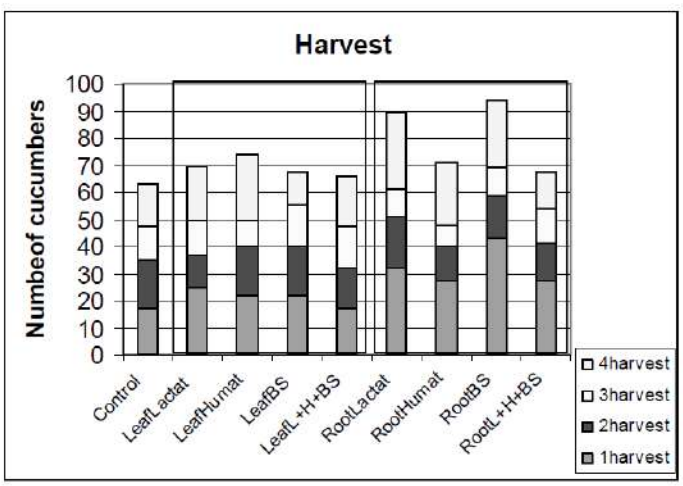

Fig. 6. Effect of application biostimulators (Lactate, K-Humate, *Bacillus subtilis*) on leaves and roots respectively on number of marketable fruits in four harvesting periods of 9 days each.
No significant differences

5. Biological Control Methods in Plant Protection

Effect of pine, castor oil and humate against the larva and adults of white fly (*Trialeurodes vaporariorum*)

Humates, as already described can accelerate the plant-growth, stimulates the process of the formation of plant-organs, increases the unspecific resistance of the plants against stress-conditions like to high temperature, frost, drought, strong radiation (Böhme *et al*., 2008). Humates have an influence on the nutrient-uptake and the respiration-process, the amount of sugar and amino acids, further reduce the accumulation of nitrate and make the plants resistant against diseases and viruses (Levinsky 1996; Hoang, 2003; Böhme *et al*., 2005).

Castor oil from the seeds of the castor plants (*Ricinus communis* L.) contains 90% ricinoleic acid, also 6.8-9% oleic acid and 1.4-3% linoleic acid. Furthermore, this oil contains the highly toxic ricin and the insecticide ricinin. The oil of castor seeds is belonging to the so called 'dry' oils, therefore it can be used also in medicine and for technical purposes.

Pine oil (*Pinus sylvestris*) means essential oil distilled using pine needles, and branches with a diameter until 2.5cm. For steam distillation the material will be chopped and then pressed in a specific vessel with a steam pressure of 0.5 to 0.7 MPa the distillations are finished after 5 to 6 hours. In the needles the oil concentration is about 75%, in the thin branches 17% and in thicker branches ca. 10%. The composition of the pine oil is in average as follow- α- Pinen 46%, Camphen 3%, ß- Pinen; ß-Myrcen 28%, Limonen 8.6 and Ocimen 3.5% (Georgiev, 1995).

The aim of the research was in substrate culture of tomatoes (*Lycopersicon esculentum* Mill.) in greenhouse to investigate the effect of organic plant regulators and plant oils, and the interaction of some of the treatments against white fly.

In this experiment, the population of white fly was increasing very early starting middle of February. The greenhouse temperature rose up to 24 °C, therefore, already middle of March were observed more than 20 larvae per leaf.

As treatments against white fly (*Trialeurodes vaporariorum* WESTWOOD, 1856) the humate from the company 'Humintech' as well pine oil as castor oil were used in a tomato cultivation system using pots under greenhouse conditions. The application of the treatments started after larvae density was observed with 19 to 22. The number of larvae per leaf increased very fast as well in the Control as after treatments of humate (Fig. 7).

It was distinct that humate had no influence on the development of the white fly larvae. In difference, castor oil and pine oil had significant toxic effect against the white fly larvae.

Whereas, the effect against the larvae with pine oil treatments was higher than with castor oil.

Sengalevich *et al*., (2002) had similar results in his research using pine and castor oil against the larvae of Colorado beetle with about 70-80% mortality of the larvae.

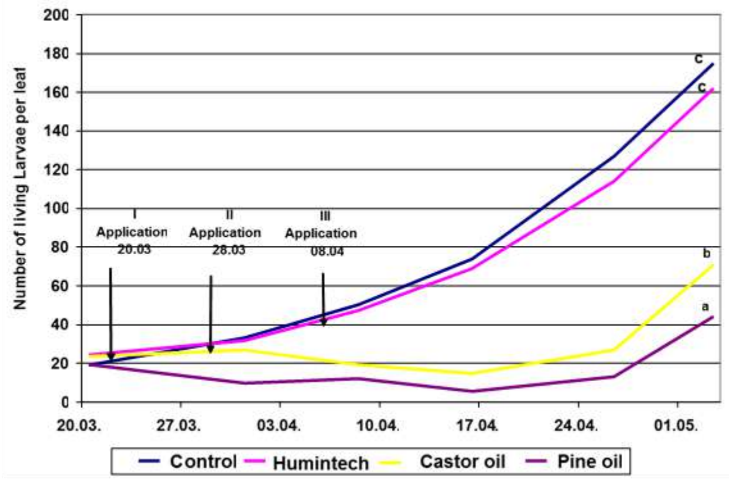

Fig. 7. Number of living larvae of T. vaporariorum on tomato plants in greenhouse after 3 times application of Humate (Humintech) (0.05%), castor and pine oil (1%)
(Different letters indicate statistical differences (P=0.05 Tukey-Test)

Furthermore, was analysed the influence of the white fly population density following the quantity of living larvae after treatments with humate, castor and pine oil on the yield of tomatoes cultivated in greenhouse (Fig. 8). The tomato yield was reduced because the high white fly population and the damaging activities of the adults and the larvae. The humate was not disturbing the white fly, therefore no significant difference could be determined in comparison to the Control.

The tomato yield in the variant with castor oil application was higher, but the difference to the Control was also not clear significant. The highest tomato yield is visible for the treatment with pine oil, this result is significant different to the other variants. In this experiment was obvious a strong correlation between the white fly population (quantity of living larvae per leaf) and the yield. On the other hand, this is showing the strong yield decreasing danger of the white fly in greenhouses.

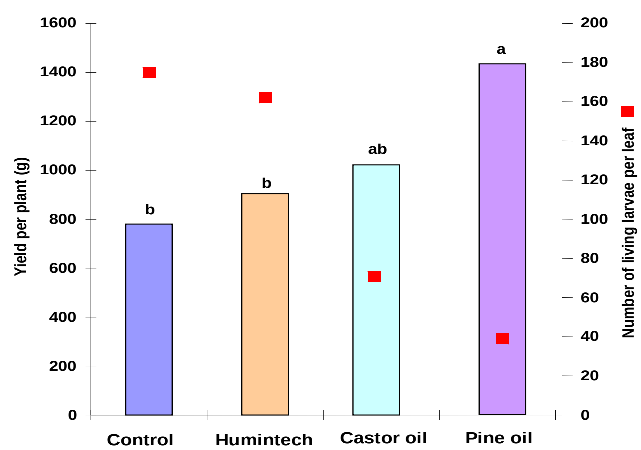

Fig. 8. Correlation between the effect of Humintech (0.05 %), castor oil and pine oil against the quantity of living larvae of *T. vaporariorum* and the tomato yield, cultivated in greenhouse
(Different letters indicate significant differences (P=0.05 Tukey-Test)

6. Soilless Cultivation Methods – Aquaponics

The use of hydroponic systems that means soilless cultivation systems were already described, for water spinach and potato minituber as well as tomato and cucumber cultivation in substrate culture. There are many new innovations in hydroponics, here will be shortly described some results with the Aquaponic system, also because Asian leafy vegetables were cultivated together with Tilapia fishes.

In many South- and South-East Asian countries, aquaponic is common practice in particular in the rural areas. The combination of aquaculture and vegetable production is for subtropical and temperate regions the right way and basis for the innovative idea to combine aquaculture and hydroponic systems and starts already 40 years ago (Naegel, 1977; Drews and Renner, 1992).

During this time are visible different investigations concerning species of fishes, and vegetable cultivated but also technical and biological systems (Rakocy *et al*., 2006; Trang *et al*., 2010; Nichols and Savidov, 2012). Aquaculture and aquaponics have different problems in particular regarding the nitrogen content in different forms (ammonia, nitrite and nitrate) (Wongkiew *et al*., 2017). The commercial aquaponics are mostly recirculating systems, were the water from the fishes is used to feed the vegetables or herbs. Two systems were compared in these investigations, the recirculating aquaponic system with the fish and plant culture in aquaculture, as well as a hydroponic system.

Asian herbaceous plants used in hydroponic and aeroponic system

Two Asian species are selected to be used in hydroponic and in aquaponic systems because they are well adapted on high water temperature and permanent rooting in high amount of water in nutrient-film-technique (NFT).

Water Spinach (*Ipomoea aquatica* Forssk.) or water convolvulus, family Convolvulaceae, is one of the most important vegetables in the tropics of South-East-Asia as well as in Japan, Australia and some regions of Africa. There are vining and straight growing types.

Water Celery (*Oenanthe javanica* (Blume) DC, also known as, water dropwort is a perennial herb that grows to about 1 m in height of the family Apiaceae. The young leaves or stems can be eaten raw or cooked. The taste is a little similar to leafy celery or parsley. In Korea is also used for some kind of Kimchi. The stems are single harvested by hand.

Leaf Mustard (*Brassica juncea var. integrifolia* (L.) Czern.) A leafy cabbage also known as Indian mustard belongs to the family Brassicaceae is an annual herbaceous plant. The leaves can have a length from 25 to 45 cm and are oblong, often dark green. The inflorescences can have a height of 50 to70 cm. The photoperiodic reaction can be different in temperate region mostly a long-day plant.

Fish used in aquaculture and aquaponic systems

Nile Tilapia (*Oreochromis niloticus*) is a species of tilapia, belong to the family Cichlidae native to Africa from Egypt south to east and central Africa, and as far west as Gambia, as well as native in Israel (Froese and Pauly, 2015). There is a high biodiversity about 700 species are known.

Adults reach up to 60 cm in length and up to 4.3 kg. Omnivores as the Tilapia can have after 8 to 9 months 500g weight, more often they have in aquaponics ca. 350g.

In three different experiments were investigated the use of hydroponic, aquaculture and aquaponic cultivation systems, the plant density, frequency of harvesting, composition of the organic or mineral nutrient solution and the EC and pH value and their influence on the yield and the content of minerals, bioactive compounds and vitamins.

Plant Production

In all experiments, Water Spinach (*Ipomoea aquatica* Forssk.) was cultivated in the last experiment also Chinese Celery (*Oenanthe javanica* L.). In this experiment, the NFT was improved and covered with plastic to reduce growth of algae and water losses. For Water Spinach was no significant difference observed regarding the plant height after five harvests. Regarding fresh matter (yield) could be detected significant lower FM in the aquaponics Tab. 3. The time between the harvests was for hydroponics 22.75 days, whereas for aquaponics 26.25 days in average. In case of Water Celery, no significant difference was determined for plant height and yield. It seems Water Celery is a very convenient plant for aquaponics, the only problem was the development of several stolons in the channels. Therefor another experiment was carried out were the plants grew in 8 cm pots filled with a peat mix substrate.

In case of Water celery, in 30 days cultivation time were produced 700g m^{-2} with 30 plants per m^2, Sasmitamihardja (1993), mentioned a yield of 1000g per m^2 in similar cultivation time.

Table 3. Plant Height and Fresh matter (FM) or yield, of Water Spinach (*Ipomoea aquatica*) and Water Celery (*Oenanthe javanica*) in Hydroponics and Aquaponics.

Cultivation time 4 months and five harvests			Cultivation time 3.5 months and three harvests	
Water Spinach			**Water Celery**	
	Height (cm)	Fresh matter (g plant $^{-1}$ d^{-1})	Height (cm)	Fresh matter (g plant $^{-1}$ d^{-1}
Hydroponics	37.54 a	3.77 a	34.70 a	0.77 a
Aquaponics	31.52 a	2.68 ab	35.66 a	0.80 a

Internal compounds in the cultivated plants

The content of bioactive compounds as chlorophyll a and b, carotene and vitamin C were analysed in Water Spinach and in Leaf Mustard (Table 4). Regarding vitamin C in Water Spinach, the content was in line with the results of the literature there are in a wide range of 30 to 210 mg per 100g FM (Prasad, 2008).

There are significant differences of the content of all four compounds analysed in particular in Water Spinach cultivated in aquaponics and hydroponics. It is remarkable that in Water Spinach the content of Chlorophyll a and b, Carotenoid and Vitamin C are highest if cultivated in aquaponics.

Table 4. Content of chlorophyll a and b, carotenoid and vitamin C in the leaves of Water Spinach and Leaf Mustard cultivated in Aquaponics and Hydroponics (Dewenter, 2012)

	Chl a (µg cm^{-2})	**Chl b** (µg cm^{-2})	**Carotenoid** (mg 100 g^{-1} FW)	**Vitamin C** (mg 100 g^{-1} FW)
Water Spinach				
Hydroponics	19.94 b	5.46 a	4.30 b	33.13 b
Aquaponics	31.83 a	6.85 a	5.78 a	40.40 a
Leaf Mustard				
Hydroponics	14.36 a	12.18 a	2.52 a	64,65 a
Aquaponics	14.44 a	12.50 a	2.33 a	46.92 a

Different letters indicate significant difference among treatments (Tukey-test, P≤0.05)

7. Interaction Between Light Spectra and the Internal Compounds of Vegetables

Protected cultivation in particular in plastic or net houses are also important in tropical regions in order to harmonize the climate conditions. In this context, there are some researches to test colored plastic (Gmizo *et al*., 2012) or coloured nets (Darázsi Ledóné *et al*., 2017) regarding the influence of different light spectra on the growth and internal compounds in tomatoes and hot pepper. Few previous studies have investigated interactions between light intensity as well as light spectra and content of secondary metabolites. As shown by Jin *et al*., (2011), red and green light are more effective for polyphenol production in *Chrysanthemum morifolium* than white and blue light. Despite of these studies, a comprehensive research on the effects of light spectra on the concentration of secondary metabolites in plants is still missing.

The aim of the experiments with different light spectra was to investigate the effect on the growth of *Perilla frutescens* and the content of secondary metabolites under two different colored plastics. *Perilla frutescens* (L.) Britt. (Lamiaceae), also known as Shiso, is an Asian herbaceous plant native to mountainous areas from India to China but mainly cultivated and consumed in Korea, Japan, Thailand, and Vietnam. In their cuisine leaves, inflorescences, fruits, seed, and sprouts of Perilla are used raw or cooked. In addition, essential oil distilled from the leaves is used for preparation of meals, as well as sweetening agent (De Guzman and Siemonsma, 1994). To investigate influence of modified sunlight spectra on plant development, two types of colored plastic films were used: 'Half Minus Green 248' (HMG) and 'Pale Lavender 136' (PL), produced by the company LEE Filters (UK). Both plastic films provide high transmission for red and blue light and reduce green light at different scales. These films were placed above the plants on a tunnel shaped construction (l=80 cm, h=70 cm) to avoid influence of natural light from the sides.

As result of this research was detected, use of colored plastics films in cultivation of Perilla plants in protected environments influenced the investigated parameters differently. Fresh matter (FM) of Perilla leaves was about 14 g per plant in all treatments and did not respond to the use of colored plastic films (Table 5). Dry matter (DM) was significantly higher in the control than in HMG treatment. PL treatment did not differ from both. Regarding plant height, use of colored plastic films had significant effect. Significantly longer shoots were recorded under HMG treatment (about 40 cm) and PL treatment (about 39 cm) than in the control (about 34 cm).

Table 5. Effects of natural light (control) and coloured plastic films 'Half Minus Green 248' (HMG), 'Pale Lavender 136' (PL) on fresh matter (FM), leaf dry matter (DM) and plant height of *Perilla frutescens*. Percentages indicate changes compared to control (100%).
Different letters indicate significant difference among treatments (Tukey-test, P≤0.05), n=24 (fresh matter, plant height), n=16 (dry matter)

Light Treatment	Fresh matter g/plant)	Dry matter (%)	Plant height (cm)
Control	14.91	15.51 a	33.88 b
HMG	14.27	13.77 b	40.18 a
	−4.29%	−11.21%	+18.60%
PL	13.60	15.20 ab	39.17 a
	−8.79%	−2.00%	+15.61%

As visible in Table 6, the secondary metabolites as polyphenols, flavonoids, and anthocyanin responded differently to the light conditions, although anthocyanin and flavonoids are also polyphenolic compounds. The polyphenol content was considerably higher in control plants than in HMG treatment. However, PL treatment showed no differences compared to the other two treatments. Regarding the flavonoid content there were no significant differences between treatments (Table 6), but its concentration showed negative correlation with light intensity, r=-0.85.

Table 6. Content of secondary metabolites of *P. frutescens* plants after three weeks: polyphenol, flavonoid, anthocyanin, and antioxidant activity (AOX) at natural light (control), 'Half Minus Green 248' (HMG) and 'Pale Lavender 136' (PL) treatments. Percentages indicate changes compared to control (100%). *Different letters indicate significant difference among treatments (Tukey-test, P≤0.05).*

Light Treatment	Polyphenol [mg GAE/100 g FW]	Flavonoid [mg QE/100 g FW]	Anthocyanin [mg SE/100 g FW]	AOX [mg TEAC/100 g FW]
Control	880.19 a	412.58	201.34 a	332.52 a
HMG	389.59 b	414.77	37.53 b	154.19 b
	-55.74%	*+0.53%*	*- 81.36%*	*-53.63%*
PL	559.31 ab	458.20	114.19 ab	263.33 ab
	-36.46%	*+11.06%*	*-43.28%*	*-20.81%*

Both plastic films investigated here reduced light intensity and changed light quality as well.

Although the light intensity was reduced considerably, FM and growth were not adversely affected by the use of these coloured plastic films. However, the content of secondary metabolites was in some cases negatively affected, especially by the use of HMG plastic film. The fact that the film with higher light transmission (HMG) affected the secondary metabolites of Perilla more negatively than the less transparent film (PL) indicates the importance of light quality for accumulation of secondary metabolites.

Conclusion

For supply of the increasing population in South-East-Asia with horticultural commodities and in particular vegetables and fruits, strategies for sustainable production are of high importance.

- In order to develop sustainable strategies for the production of horticultural crops, evaluation of sustainable land use in the different regions is recommended. Sustainable production of horticultural plants can be realized only in a 'bottom up' approach. That means the farmers have to be convinced in sustainable action and to be involved in selection of the appropriate criteria in frame of the economical, ecological and social aspects.
- A complex multicriterial analysis can show the strengths and weaknesses of the current land use in the regions and can help to develop strategies for a sustainable farming system.

As stated, it could be shown in two examples the advantages of using in vitro methods as better propagation method. In this regard were successfully described two examples for using in-vitro methods breeding of water spinach (*Ipomoea aquatica* L.) and propagate potato (*Solanum tuberosum* L.) in order to receive minitubers as seed potatoes.

- The water spinach seed material had a very low germination rate on the other hand exhibited a high variability that could be shown in vitro concerning callus formation and multiplication rate as well as in greenhouse evaluation. This variability could be used for selection of stable clones for greenhouse production of water spinach in a temperate climate. The seven clones presented here could be characterized and clearly distinguished by agronomical and botanical parameters. So far these clones can be only propagated vegetative by cuttings or micropropagation.
- Minituber production of potato by using in vitro technique and aeroponically systems was successful. It can be concluded that plant density is an important parameter for the

efficiency of minituber production. The plant density for potato propagation in an aeroponically system could be reduced considerably (up to 25 plants per m²). High tuber productivity of 1100 minitubers per m² was gained in about 4 months of cultivation in after in vitro in total.

As in former experiments it was again visible the positive effect of biostimulators as humic acid, lactic acid and *bacillus amyloliquefacien* (subtilis) as single applications or there combinations.

- It was visible, that all biostimulators had in most cases a stimulating effect on shoot development and number of marketable fruits.
- All substances tested stimulated the vegetative growth while yield was higher if LACTOFOL and Bacillus subtilis was applied.
- Root application of biostimulators were more effective as the leaf application, in particular if Lactate or Bacillus subtilis was added to the nutrient solution.

Nowadays biological control of pests becomes more important. Beside use of antagonistic insects, there are traditions to use plant oils.

- The plant oils from castor and pine (1%) were showing an insecticide effect against the larvae and adults of white fly (*T. vaporariorum*).
- It could be observed a significant higher effect of pine oil against the larvae of white fly. Whereas, the effect of castor oil was significant lower, but higher than the humate from Humintech.

Aquaponics can be used successful as closed recirculated system for production of leave vegetables. Completely nutrient supply using only fish water as organic fertilizers can secure satisfying yield.

- The yield with leave vegetables was different, on such conditions well adapted Water Spinach and Water Celery had mostly higher yield in aquaponics, whereas the yield of Leaf Mustard was higher in hydroponics.
- It was determined a positive influence of aquaponic growth condition on some internal compounds as the bioactive ingredients. The content of chlorophyll a and b, carotenoid and vitamin C in the leaves of Water Spinach was significant higher in aquaponics.

The great market value of Perilla and rising consumer interests in healthy food could require its cultivation in regions where the light intensity is a limiting factor for growing Perilla.

- Both plastic films (HMG and PL) reduced light intensity and changed light spectra as well. Although the light intensity was reduced considerably, FM and growth were not adversely affected by the use of these coloured plastic films.
- The plastic with higher light transmission (HMG) affected the secondary metabolites of
- Perilla more negatively than the less transparent film (PL).
- Although the used plastics did not show any positive effect for the production of Perilla, use of coloured plastics should be considered regarding shade avoidance response.

In further investigations coloured plastics with higher light intensity transmittance, lower transmittance for green and far-red light.

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Integrated Vegetable Pest Management in the Tropic

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Abstract

Tropics regions have favourable climate which is suitable for crop cultivation throughout the year since they have specific characters e.g. high mean of annual temperature, high amount of annual rainfall, and highest amount of annual solar ray. Unfortunately, this specific climate also responsible for predominance of pests and crop diseases in the areas. With the various cultivation problems in the most of developing countries in the tropic, such as limited funds, infertile soil, lack of irrigation in the arid areas, and poor mechanization system, pest and disease control become more challenging. Thus, smart pest and disease management strategies should be implemented to manage pest and disease attacks in such situation. The current article aims to review some conceivable vegetable pest and disease management strategies in tropical areas.

Keywords: tropic, pest and disease management

Introduction

The tropical zones are defined as the regions that situated in the nearby equator, between the parallels 23°27' south of the equator (Tropic of Capricorn) and 23°27' north of the equator (Tropic of the Cancer). With the notable features, i.e., high absorption but low release of heat, various organisms could be easily found in that areas including lianas, epiphytes, various trees, arthropods, reptiles and many more (Schnitzer and Carson, 1999). The diversity and the richness of tropical and subtropical pests is higher than temperate pests. Climatic and microclimatic known to affect pest biology and status (Stejskal, V and Alois, H. 2002).

In addition to its importance as key micronutrients sources (Dias, 2012), vegetables that commonly grown di tropical regions also provide additional income for farmers whom usually only have an average farm size <2 ha (Johnson *et al*., 2008). However, vegetable productions in the tropic areas face major biotic and abiotic limitation factors, for example: high disease incidence due to its yearly high rainfall (Bowen and Kratky, 1982) and high pest population, infestation and establishment due to favorable condition such as heavy rain and dews, warm temperatures, and dry climates in that regions (Landston and Eaker, 2009). The understanding of the pest populations and their natural enemies' population in the tropics regions is more difficult due to climate change impact on the changes of pest status, pest invasion, migration, increasing diseases or insect populations (Cilas, 2016).

Chemical control is commonly applied by farmers to manage pest population and to get higher income (Gerken *et al*., 2001). Unfortunately, overuse pesticides have been lessening chemical control efficiency. It is known that, population of insecticide resistant pests in the tropic is higher than in the tropical and sub-tropical regions compare to those in temperate areas (Stejskal, V and Alois, H. (2002).

Almost 60 years ago, negative impact of overuse pesticide elicited emerge of integrated pest management (IPM) concept (Kogan, 1998). IPM is ecological based strategy to effectively manage pest population while minimizing the possible negative impact both to people and environment by using combination of various pest control practices (Ehler, 2006). The development of IPM strategies in tropical regions of South East Asia was triggered by the extensive rice brown planthopper outbreak due to the overuse pesticides application during 1970s to 1980s. In 1980s, Farmer Field Schools (FFS) is one training program that supported by FAO and that was developed to educate farmers based on their participatory experiential learning (Kenmore, 1997. FFS have disseminated IPM to rice farmers in the numerous countries, such as Philippines, Indonesia, Sri Lanka, Bangladesh, India, Thailand and Malaysia. IPM-FFS was first initiated in Indonesia in 1989 for rice farmers, but later it applied for vegetable, cotton and other crops production system. Following the successful FFS implementation in Indonesia, IPM-FFS programs were then held in other 12 Asian countries and implemented in Africa, Latin America, the Middle East and Eastern Europe (van den Berg and Jiggins, 2007). The current article aims to review the main tactics used in IPM implementation in the tropic areas, particularly in tropical South East Asia.

Integrated pest management (IPM) strategies

Integrated Pest Management (IPM) strategies that could be applied in managing pests and diseases are crop manipulation/bio-engineering, pest/pathogen disruption, ecological understanding and engineering, time management, and decision support system.

IPM: crop manipulating or bioengineering

Crop manipulation or bio-engineering could be described as efforts to enhance crop growth and improve crop ability to overcome biotic and abiotic stresses. Crop manipulating could be done through the use of high quality seed and seed treatment (Mancini, 2014); resistant rootstock (Guan and Zhao, 2012) or resistant varieties (Dhaliwal *et al*., 2004); inducing plant resistance and growth (Najam, 2017); managing plant nutrition need to improve its vigor (Dordas, 2007); applying plant hormone (Denance, 2013); and utilization of a-virulent/weak pathogen (Ricci *et al*., 2016).

IPM: pest and disease disruption

Pest and disease disruption tactics could be described as efforts to prevent pest from attacking target crops or to reduce the impact of pest attack on target crops. The goals of these tactics include specific space and time arrangement to lessening crop availability to pests, particular interference activities to disturb pest preference on crop target, manipulating pest' habitat to increase the ecosystem diversity, and applying natural enemies to reduce pest' existence (Zehnder *et al*., 2007).

The pests and pathogen disruption could be done using various traps (Epsky *et al*. 2008), biological control (Fravel, 2005), physical control (Vincent *et al*., 2003), pesticide use (Dhawan AK and Peshin R, 2009), crop rotation, mechanical/eradication, farm location selection, transplanting or harvest time manipulation, farm sanitation, various cropping system, and mulching (Zehnder *et al*., 2007). Those tactics could be implemented individually or together (Vincent *et al*., 2003) depending on the field situation and should be applied based on effectiveness of the selected method and risk evaluation prior application (El-Shafie, 2018), since each method has different control mechanism. In reality, however, pesticides were used more frequently than other pest management tactics (Vincent *et al*., 2003). Pest control using pesticides should only be applied when the pest populations is high and might causing economic damage. Further, only selected pesticides should be properly used to minimize negative impacts on the environment and to avoid the pest' resistance and resurgence (Dhawan and Peshin, 2009).

The lack successful application of other tactics made pesticides used become most preferred pest control tactic. Michaud (2002), for example, mentioned that classical biological control has limited power to suppressing invasive pest species populations. Although, the use of biological control in the tropic is promising, due to the large tropical diversity that is good source for natural enemy conservation, Fravel (2005) emphasized that successful biological control could be achieved only by combining this method with other control method.

Pest disruption using various trap types are applied based on pest's response to light (light traps) which is effective for nocturnal pests; to odor (repellent, attractant, pheromone traps); to color (yellow-sticky trap); combination traits (trap crop/barrier). The use of traps baited are intended not only for disrupting pest orientations and controlling pests, but also can be used for monitoring pest population and eventually could be used to make control action decision (Epsky *et al*., 2008).

IPM: ecological engineering

Ecological engineering is an effort to manipulate ecological situation becoming unfavorable for pest and disease growth and development. This tactic includes the use of different cultivation approach, implementation of physical approach, conducting field sanitation, the use of agronomic approaches and cropping system (Hill, 2008), and applying protected farming system (rain shelter, net/green/plastic/glass house) (Jensen and Malter, 1995; Sabir and Singh, 2013). In her review, Weintraub (2007) mentioned that although population of some pests increase, but properly implemented physical barrier is useful method in reducing many major open field pests of sweet pepper grown both in tropical and subtropical areas.

IPM: time management

Time management is one of important strategies of IPM. Various tactics could be implemented in order to arrange planting and harvesting crops, for example: implementing planting calendar to increase window of pest and disease incompatibility, shortening the crop life period to reduce crop expose against pests and diseases (Sorensen *et al*., 2016).

IPM: decision support system

The successful implementation of integrated pest management will be highly dependent on the decision support system (DSS) applied in the crop production. This system is important decide "what, when, and how to do" pest and disease control. Integrated pest management DSS comprise monitoring activities (Barzman *et al*., 2015; Flint and Van den Bosch, 1981) for pest population, disease severity, pesticide resistance, pest resurgence, and pesticide impact to environment.

Further, economic injury level (EIL) (Luckmann and Metcalf, 1975) that is the smallest number of insects (amount of injury) that will cause yield losses equal to the insect management costs also become important part of IPM DSS. Additionally, IPM DSS include economic threshold (ET) (Luckmann and Metcalf, 1975) i.e., the pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level. The use of ET and EIL in pest management decision is situational. It highly dependent on crop types, its ecology, pest/pathogen categories, cost of control and price of product.

In the tropic country, such in Indonesia, where the increase of invasive pest population and diseases attacks due to the favorable climate is apparent, while in another hand pest management is challenging because the limited land ownerships and the capital available for crop production, the development of semiochemical-based tactics in area-wide IPM become the promising approach (El-Shafie, 2018). In the area-wide IPM system, the implementation of combination of various different control tactics should be organized by farmers group or organizations rather than by individual farmer, otherwise total pest population control will hardly to achieve (Hendrichs *et al*., 2007; Knipling, 1980).

Conclusions

Manipulating pest and disease biology and ecology by applying single or multiple innovative IPM tactics in the wide area perspective IPM implementation is important for effective and successful tropical pest and disease management.

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Nitrogen Management and N Use Efficiency in Tropical Field Vegetable Production

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Abstract

Intensive field vegetable production is an economically very important sector in modern day agriculture. It is characterized by very intensive inputs and often low nutrient use efficiencies and high losses, notably of N. This is also the case in the tropics, where nutrient use efficiencies tend to be even lower due to inadequate crop rotations, high pressure of pests and diseases, and lack of analytical facilities and of efficient extension channels. The basis for any sound N fertilization system in vegetable production is the drawing up of a N balance. The main term in this balance is the N mineralization from soil organic matter, organic manures and crop residues, and it is still a challenging task to quantify this mineralization. We discuss a number of fundamental questions related to the N mineralization from these different sources. It is equally important to look at more holistic factors that influence N use efficiency, including the overall soil quality (which governs N release and N loss processes) and the type of crop rotation. These factors lead us to conclude that farmers should adopt a long-term holistic view on soil fertility and N management, certainly when technical background and access to analytical facilities is limited. Central in this long-term holistic view should be the improvement and maintenance of soil quality, which requires long term sustained efforts from the farmer, but also a willingness to pay fair prices for agricultural produce.

Introduction

Field vegetable production is estimated to occupy about 1.1% of the world's total agricultural area only, but measured by its economic importance and income generation for farmers, it is probably the most important branch of agricultural production. Global fruit and vegetable production have experienced a remarkable increase. Output has been growing at an annual rate of about 3 percent over the last decade. In 2011, more than 1 billion tons of vegetables were gathered throughout the world. The global vegetable market is still predominantly a local market, with only 5% of the vegetables grown that are traded internationally. There is an increasing share of vegetables grown in protected culture in greenhouses, and in vertical farms. However, this paper focuses on open air field grown vegetables. Because of the very large potential added value, certainly as compared to arable crops, vegetables are mostly grown very intensively, and available production factors of a farm are often concentrated in the vegetable fields. This often leads to high yield levels, but with negative impact on product quality and environment. The factor most determining vegetable production is undoubtedly the nitrogen (N) availability for the crop.

Because of its overriding influence on crop production, many vegetable farmers have started to view N fertilizer application as an insurance against potential yield loss and compensation for other factors limiting production. This has resulted in often very low N use efficiencies, N accumulation in soils and high N losses. This paper focusses on a number of reasons behind this (lack of proper) N management, and discusses potential solutions, both on the short term and the long term.

Examples of N use efficiencies and associated problems in different parts of the world

Western Europe

Agriculture in many parts of Western Europe is very (input) intensive. While this is true for crop production in general, it is especially the case for vegetable production. Often areas of intensive vegetable production coincide with areas of intensive livestock production, as e.g. in The Netherlands, Flanders, Denmark, Brittany (France), etc. Traditionally, much of the livestock manure has been concentrated in vegetable production fields, and combined with mineral N fertilization, leading to an excessive build-up of soil fertility, high N mineralization potential, and high N losses. The areas mentioned above all have large positive N balances and N losses, leading to high N concentrations in ground and surface water. The N use efficiency in intensive vegetable crop production, typically less than 50%, is far lower than for arable crops. It is not uncommon for vegetable crops to receive a total N input of 300 kg of N ha^{-1}, greatly exceeding the typical N removed by marketable yield. Monitoring of residual soil mineral N following harvest of field vegetables revealed excessive values of up to 900 kg mineral N ha^{-1} (Fig. 1) [1], most of which is lost during the autumn and winter leaching period. At present the vegetable sector is under strong pressure from the European Commission to reduce its impact on the environment, by notably reducing nutrient inputs.

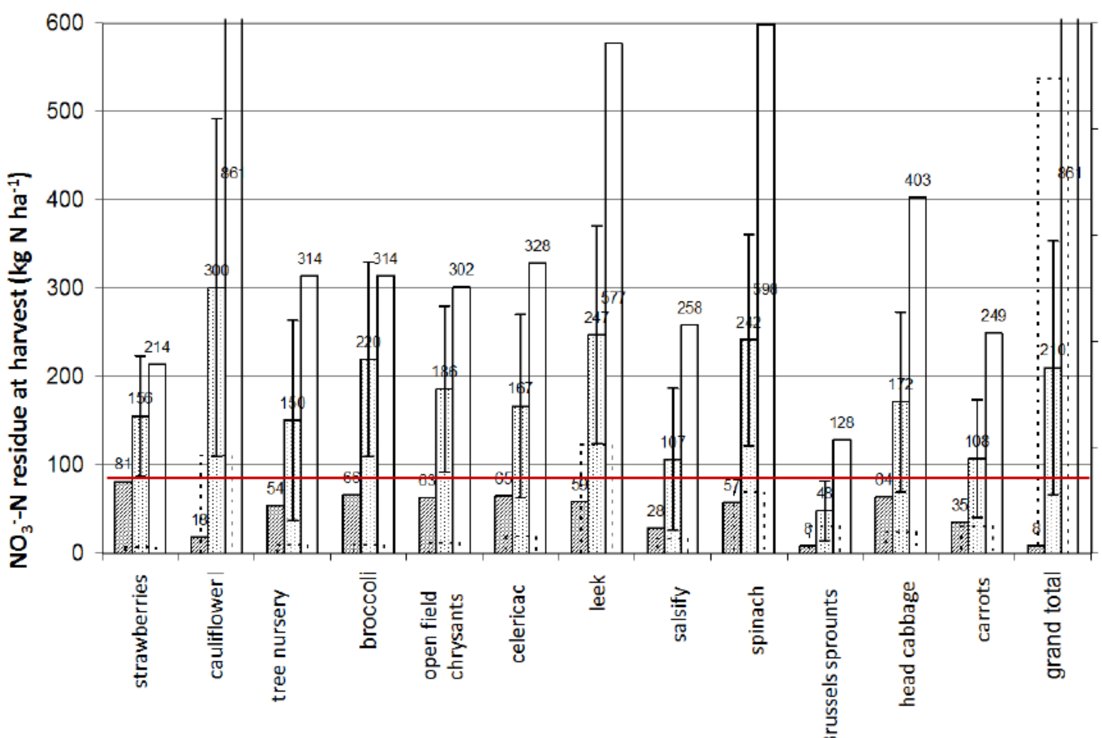

Fig. 1. Nitrate-N residue in the soil (0-90 cm depth) at or shortly after the harvest of a number of vegetables in Belgium

East Africa

Arable crops in Africa often receive very little or no fertilizer inputs, including of N. Farmers, especially close to the cities, tend to concentrate the little fertilizer and manure they have for the vegetable crops. This often also leads to low N use efficiencies and to high potential N losses, even in this situation of low overall resource availability, as shown for Burkina Faso by [2]. As an example, for East Africa, [3] calculated agronomic N efficiency (ANE) and apparent N recovery (NR) for Chinese cabbage and Amaranthus in smallholder fields in the Morogoro area, Tanzania, comparing control (unfertilized) plots to plots receiving typical doses of 200 and 300 kg N ha^{-1}.

The NR of the fertilizer applied to both vegetables was between 5 and 17% only (table 1 for Chinese cabbage), and this low value was attributed to the large N mineralization potential of the soils, leading to relatively high yields in the control treatments. This points to a potential loss of no less than 80 to 90% of the applied N, which was confirmed by the negative impact of vegetable farms on N concentrations in the surface water.

Table 1. Chinese cabbage yield (ton ha^{-1}), total N uptake (kg ha^{-1}), agronomic N efficiency (ANE) (kg dry matter kg^{-1} N) and apparent N recovery (NR) (%)

	Yield									
	Fresh matter (t/ha)		Dry matter (t/ha)		Total N uptake (kg/ha)		ANE (kg dry matter/ kg N)		NR (%)	
Days after planting	30	44	30	44	30	44	30	44	30	44
Control 0 N kg/ha	5.3^{b}	30.4^{b}	0.3^{b}	1.3^{b}	19^{b}	48.5^{b}	-	-	-	-
CHM 200 N kg/ha	10.2^{a}	50.8^{ab}	0.5^{a}	2.5a	29.3^{ab}	83^{a}	1.1^{a}	5.8^{a}	5.1^{a}	17.2^{a}
CHM 300 N kg/ha	10.8^{a}	62.6^{a}	0.6^{a}	2.9^{a}	32.6^{a}	84.6^{a}	1.0^{a}	5.1^{ab}	4.5^{a}	12^{b}
CAM 200 N kg/ha	8.8^{ab}	48.0^{ab}	0.5^{a}	2.2^{ab}	25.7^{ab}	66.2^{ab}	0.9^{a}	4.2^{b}	3.3^{a}	8.8^{bc}
CAM 300 N kg/ha	9.9^{a}	48.8^{ab}	0.5^{a}	2.2^{ab}	31.1^{a}	62.5^{ab}	0.8^{b}	3.0^{b}	3.0^{a}	4.6^{c}

Means with the same letter in columns are not significantly different by Duncan's Multiple Range Test comparison (DMRT) α = 0.05. CHM, chicken manure; CAM, cattle manure

Southeast Asia

In East and Southeast Asia agriculture has become extremely input intensive, in particular also in vegetable production. This has led to soil pollution and degradation of soil quality and excessive nutrient losses (e.g., [4]). In a field study in Central Java, Indonesia, carried out between 2006 and 2008, a number of intensively managed vegetable fields were monitored and N balances were drawn up by [5]. These N balances revealed that the six vegetable crops (Leek-potato-potato-leek-potato-cabbage) in a 2-year rotation received a total N input of up to 3770 kg N ha^{-1}, whereas actual yields were low due mainly to pest and disease incidence, leading to an overall N surplus of up to 3800 kg N ha^{-1} and a N use efficiency of as low as 12%. Clearly this is a massive wastage of fertilizer inputs, and poses serious threats to the environment through all kinds of N losses, notably leaching losses.

These examples demonstrate that N fertilizer indeed is viewed by farmers as insurance against potential yield loss, but often bears no relation to actual crop needs, mainly due to relatively low N fertilizer prices and a lack of knowledge with farmers. The solution to this problem should thus come from an increased technical knowledge on N fertilization, an understanding of the importance of soil quality for efficient crop nutrition, a genuine concern about environmental issues, all of which requires well-functioning extension services and reliable fertilizer recommendation systems.

Table 2. Long term N balances and N use efficiencies in intensive vegetable production over 6 cropping seasons (2 years) in Central Java, at 2 locations (Wonosobo and Kopeng).
IP: improved practices; FP: farmers' practices

Locations/ Treatments	Long-term N-balance			N efficiency		
	kg ha^{-1}			%		
Wonosobo	Nurhakim	Sudarto	Sucipto	Nurhakim	Sudarto	Sucipto
IP	1885	1185	627	21	25	21
FP	3314	2232	962	12	14	15
Kopeng	Ngatemin	Nano	Lukas	Ngatemin	Nano	Lukas
IP	1424	1016	884	28	33	42
FP	3808	1077	1370	14	30	29

N balances as basis for fertilizer advice and tool for awareness raising

Fertilizer recommendation systems, in particular for N, exist in many forms of highly varying complexity. The simplest (but perhaps least accurate) are the fixed recommendations, irrespective of soil type and other factors. At the other end of the spectrum are computer simulation models that incorporate much of the mechanistic knowledge on plant and soil processes and that are potentially highly accurate. However, their use in practice is limited by the data requirements to run the models. N fertilizer recommendation in practice is best based on an assessment of the major factors that may influence N availability during the cropping season, in a simple but solid framework called a N balance (sometimes called balance sheet methods). Such balance can be written as in table 3, representing the different components of N demand and N supply.

Table 3. The different components of a N balance

N demand		N supply
N uptake by the crop		Nmin in soil before fertilization
+		+
Residual soil mineral N	=	N mineralization
+		+
(estimated N losses during growing season)		N fertilization

Such a balance will yield a N fertilization advice as:
N fertilization = N uptake by the crop + Residual soil mineral N + (estimated N losses during growing season) – Nmin in soil before fertilization - N mineralization

Drawing up this balance requires both soil analyses and estimating mineralization processes. A soil analysis is common practice in Western Europe, but very rare in tropical environments.

Provided that soil analyses are feasible, the main challenge in drawing up such a N balance lays in the estimation of the different components of N mineralization. Indeed, farmers usually have a good idea about expected yield (and may derive from this the expected N uptake), whereas residual soil mineral N at harvest (and possible N losses during the growing season) should be kept as small as possible, and initial soil mineral N before fertilization can be measured. The estimation of these terms thus requires some basic knowledge with farmers on N dynamics in soil. Estimating the N mineralization from different sources, however, is still a matter of very active scientific research, and remains difficult, especially in systems with large organic matter inputs and highly variable crop rotations, typical of intensive vegetable production.

N mineralization from native soil organic matter and added organic materials

N mineralization from native soil organic matter

The most difficult component of N mineralization in soil is the mineralization derived from the native soil organic matter (SOM), i.e., the organic matter that results from the stabilization of organic material applied in the near or distant past, and that is determined largely by climate, soil type, and management practices. For a crop rotation and management that does not change much over time, farmers can tell almost by experience what mineralization they expect from SOM. In such rotations there are relatively few issues with N efficiency and N losses. However, in intensive vegetable production, farmers apply large amounts of both mineral N fertilizer and organic materials, till the soil intensively, and grow many different crop species. All mineral N that is not lost but immobilized as organic N, and all organic N applied that is not taken up or lost but still present in organic form, contribute to the mineralization potential of the soil.

N mineralization from native SOM is built in in various N fertilizer advice systems, and is usually based on routinely analyzed soil parameters, such as SOC, SOM, SON. Often, a fixed factor is used to derive the expected yearly N mineralization from these parameters. However, such simple systems are typically usable mainly in traditional arable rotations and much less in intensive vegetable rotations, for the reasons mentioned above. Much more sophisticated parameters and combinations have been used to try to predict N mineralization in such soils, but with very limited success. Recent examples of research to isolate kinetically different soil N fractions include the combination of soil physical and chemical fractionations (e.g., [6]) and of subcritical water extraction [7]. However, no methods have found broad applicability because correlations with field-measured N availability are often low [8].

Alternatively, N mineralization can be measured in the laboratory or in the field, but such measurements are exceedingly time consuming and costly and thus have no relevance for practical advice for farmers.

The only realistic way to get a good idea of the N mineralization potential of a soil is to evaluate the long-term management history of a field, and try to evaluate its repercussions on N mineralization. If there have been frequent and large applications of organic manures and residues in the past, one can expect also a large residual effect, i.e., high N mineralization rates. This requires a good bookkeeping by the farmer and a highly professional attitude, which is hampered by a lack of educational level of many farmers in the tropics, or by the fact that farming is only a part time activity. Alternatively, farmers may have their soils analyzed for mineral N content at the end of the vegetable growing season. Provided that other factors were taken duly into account at the time of fertilization, high values of residual soil mineral N probably point at an underestimation of N mineralization from native SOM. Farmers can then reduce the fertilization in subsequent growing seasons to take this into account. However, soil sampling and analyses are relatively expensive, and may even be simply impossible due to logistical reasons.

N mineralization from added (exogenous) organic materials

Unlike for N mineralization from native SOM, there are some generally accepted rules of thumb that can be used to assess the contribution of added (or exogenous) organic materials to N mineralization. Probably the best-known parameter for this purpose is the C-to-N ratio (C/N) of the organic material, with high C/N ratios (above 20-30) leading to N immobilization and low C/N ratios (below 20-30) leading to net N mineralization, when considering a single cropping season.

Alternatively, the N content of the organic material can be used, as this is very strongly (negatively) correlated to the C/N ratio. More sophisticated parameters can be used to predict N mineralization, such as lignin, polyphenols, water soluble contents, or combinations of these parameters, which seem to be particularly useful for predicting N mineralization from tropical legumes. A major challenge in predicting the contribution to N mineralization of these materials, is knowing the actual N content, which may be highly variable even amongst batches of material of the same origin. E.g., the N content in animal manure depends very strongly on the type of fodder, the age of the animals, the amount and nature of other materials added (litter, straw), the storage time and storage conditions of the manure, possible pretreatments etc. Because of this, farmyard manures may either release significant amounts of N, or on the contrary even immobilize N. Also, the N content of plant material such as green manures may differ depending on the age of harvest, the N availability in soil during the growth of the green manure, etc.

Again, it would be totally unrealistic to expect farmers to have all the organic materials they apply analyzed for N content. In conclusion, despite the fact that N mineralization would be relatively easily predictable if the N content or C/N ratio of organic materials were available, it turns out that because of the constraints on such analyses, predicting N mineralization from these materials is difficult at farmers' level.

The special case of N mineralization from crop residues

In vegetable production, often only a relatively small part of the crop is harvested as marketable yield, whereas a large part remains as crop residues (or unmarketable yield) on the field. Typical examples include head cabbages, cauliflower, broccoli, tomatoes, chili peppers, egg plants, ...

Often, these crop residues are incorporated in the field during the first tillage operations, but farmers fail to take into account the N mineralization from these residues in the fertilization for the next crop. In the case of e.g., cauliflower and broccoli, roughly two thirds of the total N uptake by the crop is in the crop residues, and these residues mineralize rapidly and completely and given their low C/N ratio, all N will be mineralized and be available to the next crop. Failure to take into account N mineralization from crop residues of vegetables has led to major issues with respect to nitrate leaching losses (Fig. 2) and high nitrate contents in surface waters in regions of intensive vegetable production in humid temperate climates [9]. Although there is a serious lack of monitoring of water quality in the tropics, it is certain that similar issues arise in surface waters in the tropics because of the same reasons. The excessive N balances in vegetable cropping measured in our research in East Africa [3] and Southeast Asia [5] indeed suggest massive N losses by leaching.

Soil quality and N use efficiency are inseparable

The efficiency of any form of fertilizer N (organic or mineral) is determined by the extent of loss processes occurring in soil. These N loss processes are very intimately linked to the status of soil quality. In that respect, the different factors determining soil quality, namely physical, biological and chemical, cannot be viewed in isolation, but always act together. Indeed, the physical soil quality is a function of the soil biological activity, and soil biological activity is determined by the soil structure (habitat space, soil aeration, water holding capacity, hydraulic conductivity). A good soil quality allows for intensive and deep rooting, and intensive and deep rooting contributes to further improving soil structure and provides energy for the soil food web. Poor soil structure and poor aeration suppress biological processes that are responsible for N mineralization. Moreover, anaerobic conditions lead to denitrification: high N fertilizer application rates, in combination with high inputs of organic materials will lead to excessive N losses under the form of N_2O and N_2. Poor soil structure resulting in limited rooting systems mean that only a very limited fraction of the total soil volume can be explored by the crop, i.e., much of the N present in the soil will not be available for crop N uptake and thus be prone to losses.

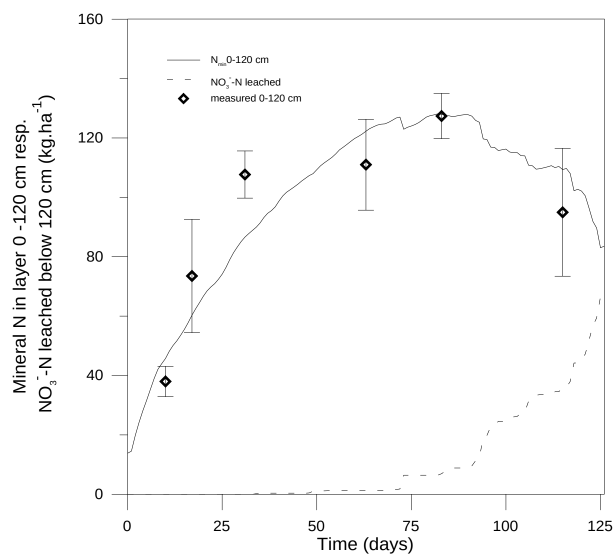

Fig. 2. Soil mineral N (0-120 cm depth) (diamonds: measured values ± st dev; solid line: simulations) and cumulative NO_3 leaching (kg N ha^{-1}) (dashed line) following incorporation of cauliflower leaves in autumn in a loamy sand soil

Soil quality in vegetable production is often low because of very intensive tillage operations, narrow crop rotations leading to build-up of diseases, and high use of pesticides that also affect non-target organisms. E.g., intensive vegetable farming, with "Western type" vegetables in Central Java started only a few decades ago, but (conventional) farmers complain that yields are declining already for years, despite ever increasing inputs of fertilizers and pesticides. We have compared soil quality of such conventional fields with that of neighboring organic fields [10]. In these organic fields, much wider crop rotations were in place, and there was much attention for the maintenance of soil quality, by the judicious application of composted organic materials and by banning all synthetic pesticides. It turned out that biological activity was massively higher in the organic vegetable fields, and that yields in the organic fields, while at a lower level than in the conventional fields at that time, remained relatively stable, in contrast to declining yields in the conventional fields.

Clearly, the message is that vegetable farmers in the tropics should pay much more attention to soil quality, given its overriding influence on N use efficiency (and N losses), and the long-term impact on yield and yield stability.

The rotations and the type of vegetables grown

The crop rotation also determines N use efficiency, in that different vegetables have different capacities of taking up available N (associated with the rooting system and with the length of the growing season). Vegetables with shallow roots and short growing period should be alternated with vegetables with deep and intensive rooting and longer growing period. In that way, the mineral N can be taken up to greater depths, and mineral N leached below the rooting depth of the shallow rooting crop can still be utilized. Often, vegetable farmers in the tropics practice very narrow crop rotations, which leads to high pressure from pests and diseases. Obviously, a diseased crop will take up less N than anticipated at planting, leading again to low N use efficiency and high N losses. In that sense, crop health and N use efficiency are intimately linked.

Also, alternating vegetables with green manure crops should be considered a must in sustainable vegetable production, given that these green manures can break pest and disease cycles, scavenge nutrients leached to the subsoil, and in general improve soil organic matter status and soil structure. This greatly improves long term N use efficiency.

“Western-type” vegetables (and fruits), and in general crops that are not adapted to the local edaphon-climatic conditions, are in high and increasing demand in many tropical countries. This concerns crops such as cauliflower, broccoli, leek, potatoes, tomatoes, etc. Because import of fresh vegetables over large differences is impossible on large scale, these vegetables are also grown increasingly in the tropics to meet the local demand. Typically, these vegetables, apart from being particularly sensitive to all kinds of pests and diseases, have a very high nutrient demand. This is in contrast to the large variety of local vegetables (water spinach, fenugreek, different types of gourds, amaranth, drumstick, ...) that thrive without much inputs of fertilizers or agrochemicals.

Profit margins are, however, much smaller for the local vegetables, and therefore less attractive for farmers to grow. It goes without saying that these Western-type vegetables represent a much greater risk for low N use efficiency, and a greater threat to soil and environmental quality at large.

This poses the fundamental problem of globalization and its impact on agriculture and environment.

The way forward

From the above, it is clear that efficient N use in intensive vegetable production is a global challenge, but all the more in the tropics, because of a lack of recommendation systems, lack of knowledge with farmers, lack of laboratory facilities for soil and fertilizer analyses, unadopted crop rotations. So, what can be done in practice to improve N use efficiency? First and foremost, farmers (and extension workers alike) should be made aware of the problem of overfertilization, notably its economic repercussions (wastage of inputs) and environmental ramifications (long term impact on water quality, eutrophication, greenhouse gas emissions and climate change).

Without this awareness, farmers will continue to look at N as a cheap insurance against yield losses. But with this awareness, what can a farmer do give the limitations on analytical capacity?

The most important is to learn by experience and by farmer to farmer transfer of knowledge and expertise. Looking at the long term is probably the most important, even more so in case of no access to analytical facilities. It is crucial that long term inputs of fertilizers do not systematically exceed the average long-term nutrient demand of the crops in order to avoid unnecessary build-up of soil nutrients and losses. For N, the match between supply and demand should be as close as possible, but overapplication is not problematic if it is done with slow release organic fertilizers (composts, green manures), and if the overapplication is compensated by underapplication at later stages.

Another crucial point is to improve and maintain general soil fertility status, especially the physical soil fertility. Indeed, given the crucial link between soil structure and soil biological processes, a good soil structure is the best guarantee for a continuous supply of N by mineralization. Degradation of soil structure on the contrary results in increasing N losses especially in gaseous form.

The main message to convey is that farmers should have a long-term view on crop production, and explicitly include soil quality as a crucial element in that view. Obviously, this requires experience and skills, and considerable time and labour investments. Such investments can only be paid off when markets (and thus consumers) are willing to pay for this, and thus offer farmers the chance to develop this long-term perspective.

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A Survey of Soil Fertility and Shallot (*Allium cepa* Aggregatum Group) Yields in West Java, Indonesia

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Abstract

Soil fertility and crop management practices are vital parameters determining plant growth and crop yield. The research was conducted to identify the relationship between soil fertility and other crop management practices with shallot yields in West Java, Indonesia. A survey of 100 shallot growers was conducted, including face-to-face interviews and soil samples were collected from their fields and analyzed using a range of standard soil test. The grower participants are from four regions; Pacet-Bandung, Bayongbong-Garut, Babakan-Cirebon and Losari-Cirebon. The results show that low pH and Olsen-Phosphorus (P) status was common for growers with lower shallot yields. Overall, growers from the Pacet-Bandung region had the lowest average shallot yields (5.4 t/ha) and the lowest average soil pH (pH 4.9), and Olsen-P (8 mg P/kg) levels compare to other growers from regions. Growers from the Pacet-Bandung region also had high yield variability ranging from 2.3 to 11.8 t /ha, minimal use of lime by these growers is likely to have contributed to low soil pH. Further research will be conducted using field trials to determine the optimum lime and P fertilizer use strategies for shallot production, which will be tailored to the soil and climate conditions specific to the Pacet-Bandung region.

Keywords: Onion, tropical farming, acid soil

Introduction

Shallot (*Allium cepa* Aggregatum group) is an important vegetable crop for Indonesia. It is used in many Indonesian food dishes as a primary seasoning and has been widely grown in the country since the 1950's. One of the essential provinces of shallot production in Indonesia is West Java.

What is distinctive about shallot production in West Java is it occurs in both lowland and highland areas with has specific challenges (i.e. those relating to the highland areas) that are likely to be different to other provinces.

The shallot production per hectare in each region (i.e., Regency) in West Java are similar on average, ranging from 8.9 to 12.5 t/ha. However, the regional averages conceal much wider underlying variability at the farmer level. There are a variety of factors that influence yield variation for individual farmers, including; climate, soil fertility, cultivars, planting practices, and pest and disease management.

There is currently limited information about the soil fertility and crop management factors influencing farmers' shallot yield in West Java, particularly in the highland areas. It is important to gain information to understand the leading causes of yield constraints better and to identify the areas in the region that have the highest potential to improve yields. This experiment aims to identify the relationship between soil fertility and other crop management practices with shallot yields in West Java, Indonesia.

Materials and Method

Four regions from West Java were chosen based on their productivity and the elevation. Two regions are located in a lowland area; they are Losari-Cirebon and Babakan-Cirebon. Meanwhile, the two regions are located in the highland area; they are Pacet-Bandung and Bayongbong-Garut.

Randomly 25 farmers were selected from each place, so there a total of 100 growers selected.

Soil analysis

Soil samples were collected from each shallot farm participating in the survey. The soil sampling used a corer to take 16 cores (0-20 cm depth) from each area. A total of 100 soil samples were collected. The soil samples were analyzed for pH, P, K^+, Ca^{2+}, Mg^{2+}, Na^+ and S. The method of measuring pH was used H_2O. The method of measuring P was depended on the pH. If the pH was less than 5.5, the method used the Bray 1 method. Moreover, if the pH were equal or more than 5.5, the Olsen method would be used. The P Bray-1 values will be converted to P-Olsen with equation Y=3.5+0.42X (Mallarino, 1995). All cations (K^{2+}, Ca^{2+}, Mg^{2+}, Na^{2+}) were extracted by 1 M Ammonium Acetate (pH 7) and analyzed by AAS method. Meanwhile, S analyzed was extracted by Morgan Venema (pH 4.8) with Spectrophotometer method.

Questionnaire

A questionnaire was developed based on the objectives of the study, exploring the association between crop management especially fertilization practices on shallot yield.

Results and Discussion

Soil Analysis

Table 1 presented yield and soil analysis result in all sites. Overall Pacet-Bandung had a lower yield also lower soil analysis values compare to other areas. That means Pacet-Bandung less fertile than other regions.

Table 1. Soil analysis results in all sites

Variables:	**Babakan-Cirebon**	**Losari-Cirebon**	**Bayongbong-Garut**	**Pacet-Bandung**	**Optimal Range**
Yield (t/Ha)	12.17	9.14	7.65	5.41	
Soil Analysis					
pH	6.3	6.8	5.1	4.9	5.8-6.3
Olsen-P (mg/L)	94	69	87	8	20-30
K^+ (me/100g)	0.9	0.8	1.3	0.6	0.5-0.8
Ca^{2+} (me/100g)	30	31	8	6	6.0-12.0
Mg^{2+} (me/100g)	1.2	1.2	1.8	2.1	1.0-3.0
Na^+ (me/100g)	0.5	1.2	0.2	0.1	0.2-0.5
CEC (%)	46	45	18	14	12-25
Base saturation (%)	71	75	60	58	50-85
Sulphate-S (mg/L)	55	150	179	114	7-15

pH

Based on soil analysis, there was a significant difference between locations with some of the soil fertility tests. Pacet had the lowest pH compare to other regions, which is likely to a contributing factor to the lower yields of participants in this region. Overall pH ranges from 4.2-7.8 across all of the sites sampled There is a general trend of lower yields for sites with the lower soil pH levels, (Fig.1). Soil with pH<5.5 had a higher number of sites with lower yields. Pacet and Bayongbong regions had lower more sites with both lower soil pH and yields. Therefore, there could have been other factors in those regions contributing to lower yields. Onion can grow well and produce a good yield on pH 5.7 or 5.8 (Mathur & Levesque, 1983).

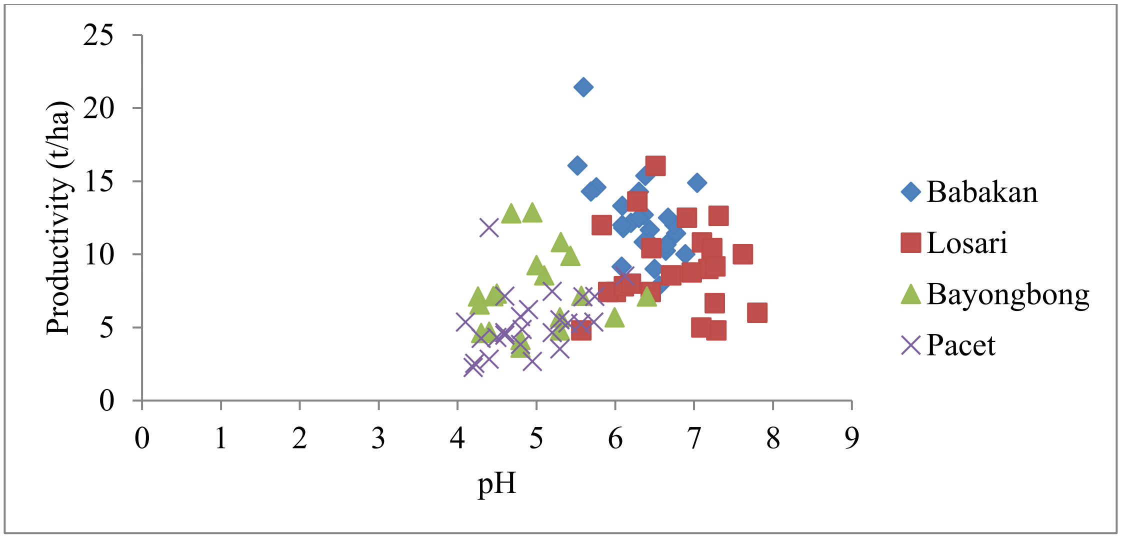

Fig. 1. Relationship between pH and productivity in each location

pH is the crucial factor in nutrients availability. In very low pH, a strongly acid soil, phosphorus will be strongly held, and the soil fertility will be very limited due to low P solubility (Foth & Ellis, 1996). If Olsen P is low, changing pH alone will not result in an optimum yield. However, in Olsen P-status was optimum, a low pH could reduce the potential yield because of root growth interference (Firmansyah, Musaddad, Liana, Mokhtar, & Yufdy, 2014). So, improving both the pH and Olsen P will increase the yield significantly.

Low pH in the soil may affect plant growth directly and indirectly. Directly, it was by inhibit plant dry weight production through root growth interference (Schubert, Schubert, & Mengel, 1990). Poor root growth at low pH is caused by a lack of net H^+ release that may decrease cytoplasmic pH values (Yan, Schubert, & Mengel, 1992). Meanwhile, indirectly affect was by the limited availability of P, Ca or Mg and by elevated Al or Mn solubility (Adams, 1981).

The lime application could improve the soil property. Liming increased soil pH, exchangeable Ca and exchangeable Mg and reduced exchangeable Al and also increase nutrients uptake (P, K, Ca, Mg and S) (Fageria, Baligar, & Wright, 1989). However, the response may differ between species. In onion, improving pH by lime was reported could increase the bulb yield significantly.

Application 2, 4 and 6 ton/ha agricultural limestone could increase pH from 5.5 to 6.0, 6.2 and 6.6 and also enhance the bulb yield from 7.6 t/ha to 22.3, 25.1 and 26.1 t/ha, respectively (Hemphill, 1985). Another work reported that the application of 12 t/ha lime could reduce soil acidity from 3.4 to 5.1 and 2.6 to 4.9 and increase the onion bulb yield from 90 g/pot to 160 g/pot and 0 g/plot to 110 g/plot (VanLierop, Martel, & Cescas, 1980).

Olsen-P

The Olsen P (mg P kg^{-1}) content in Pacet was significantly different with all areas. Pacet had a very low average Olsen P, ranged 5.82-62.79 mg P kg^{-1}. Meanwhile, the other regions had very high Olsen P-status, with average Olsen P ranging from 69.o to 94.4 mg P kg^{-1}. The higher Olsen P at sites in Bayongbong could explain why the average was higher than at Pacet even for sites will similarly low pH levels. There was a general trend of lower yields at lower Olsen P levels (Fig. 2). Pacet had the most significant number of sites with low Olsen P<25 mg P kg^{-1}, meanwhile, Losari and Babakan had sites mostly above 25 mg P kg^{-1}. Therefore, while low Olsen P is like to be contributing to low yields at many of the low Olsen P sites, there are likely to be other limiting factors.

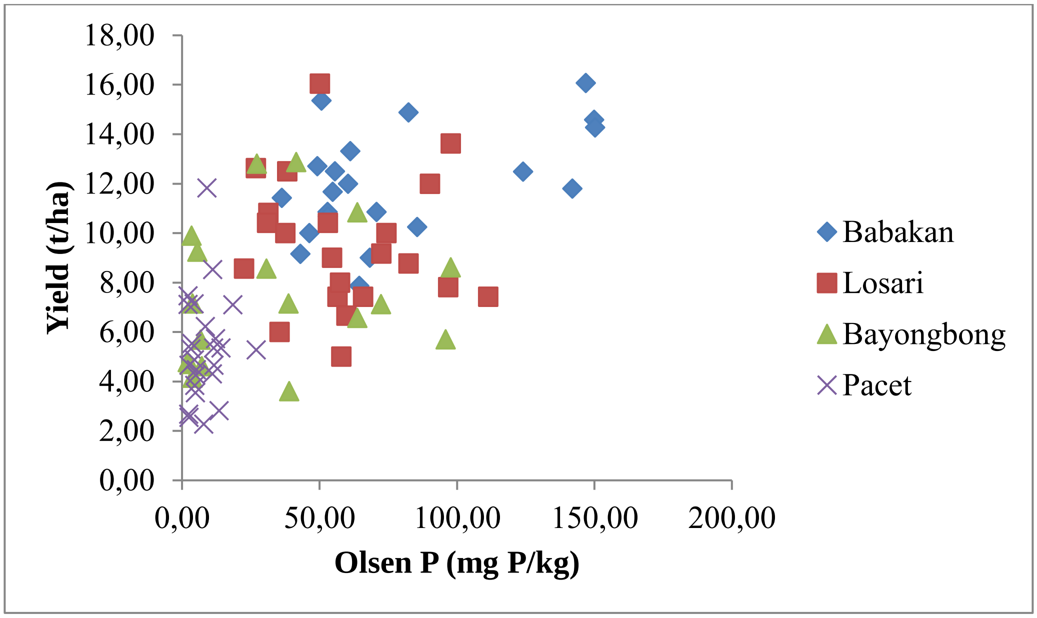

Fig. 2. Relationship between Olsen P and yield in each location

Allium has a shallow root, and this condition may lead to a deficiency of P (Sumiati & Gunawan, 2007). Phosphorus deficiencies can reduce root and leaf growth, bulb size and yield, and also delay maturation. Sumarni, Rosliani, Basuki, and Hilman (2012) reported that in very acid and low P soil condition (pH 4.3-4.8, Olsen P=6.5-17 ppm), the application of phosphorus fertilizer without liming did not improve the shallot growth significantly. Meanwhile, in neutral pH (pH=6), application of phosphorus fertilizer could enhance the shallot bulb yield.

The P-soil concentration may vary. It ranges from <0.01 to 7 or 8 mg/L, depending upon soil pH, recent additions of fertilizer P, and other soil factors. Furthermore, total P has decreased drastically since weathering. The weathering makes acid soil, and the soil fertility becomes very poor. This condition makes a limitation on plant growth because of low available P and also high levels of exchangeable and soluble Al. In most acid conditions, Aluminum toxicity is responsible on poor plant growth and low yield (Haynes & Mokolobate, 2001).

Crop Management Practice

Babakan as the highest production area had a balance lime and fertilizer rate compare to other regions (Fig. 3). Successive nitrogen fertilizer application happened in Losari, Bayongbong and Pacet. The negative impact of the high rate of N fertilizer application on dry bulb weight was have been reported by several studies (Amin, Hasan, Naher, Hossain, & Noor, 2007; Dapaah, Amoh-

Koranteng, Darkwah, & Borketey-La, 2014). Despite the high rate of N-fertilizer, Pacet farmers rare to apply lime in their lands makes Pacet soil more acid. In addition, Pacet growers mostly split P fertilization pre-planting and last stage of shallot growth. Time of fertilization is important in growing shallot. In rainy season, split application of soluble N is necessary to limit the leaching and run off of the nutrient. In contrary, split application of P fertilizer could give negative impact on shallot fresh bulb yield (Gunadi & Suwandi, 1989).In addition, Hopkins *et al*., (2010) reported that split application of P fertilizer did not enhance the P fertilizer efficiency and pre-planting application gave a better yield rather than post-planting application (Zakirullah & Khalil, 2012).

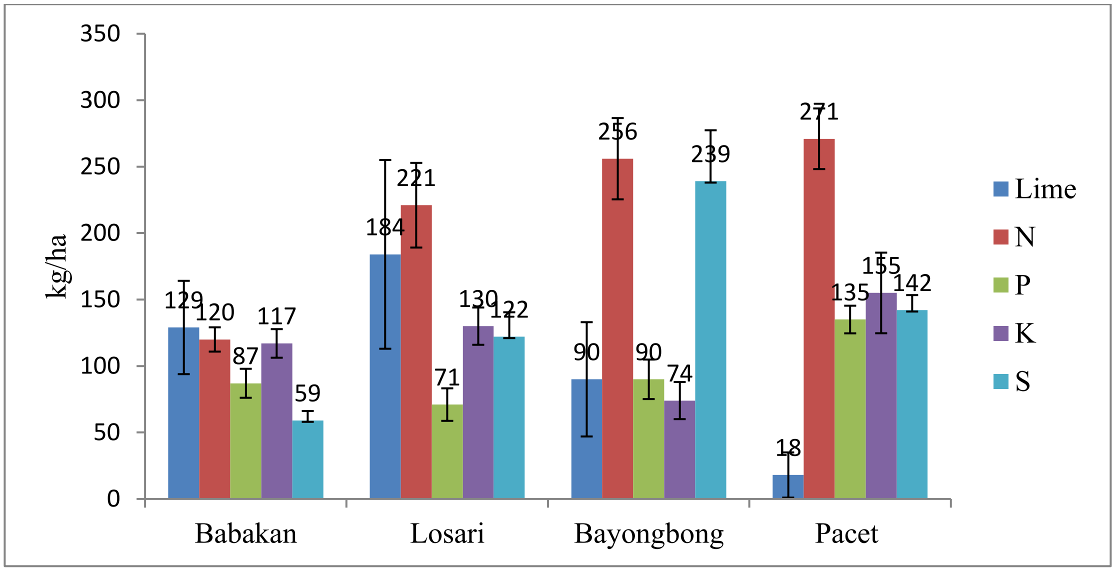

Fig. 3. The comparison of fertilizer in each location

Conclusions

There are considerable differences in soil fertility and crop management practice in identified significant shallot production site in the West Java. Pacet region is identified as a potential improvement region because it has the lowest yield and the potential causes of the low yield are pH and low Olsen-P status. Enhancing both pH and Olsen-P will improve the shallot yield.

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The Dynamic of Growth and Photosynthate Translocation of Nine Potato Varieties (*Solanum tuberosum* L.) in High and Medium Land in Central Java, Indonesia

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Abstract

The air and soil temperature differences between high and medium land lead to the differences of potato growth and tubers formation. Therefore, a research to determine the inhibition of tuber initiation and development in the field had been carried out in high (1360 m asl) and medium land (385m asl) from July-October 2016. Nine high land potato varieties were used in this study. The results exhibited that air and soil temperature between high and medium land were significantly different, which were 20.35 °C; 26.95 °C for air temperature and 21.71 °C; 28.45°C for soil temperature, respectively. Four out of nine varieties – 'Andina', 'Cingkariang', 'Margahayu', 'Olympus' – produced more than 100 g/plant tuber-fresh weight, so they were selected for further study. In the high land, the average of tuber initiation of the 4 varieties mentioned, occurred at 30 daps (day after planting) with tuber/shoot ratio was 0.726, whereas those of in the medium land occurred at 60 daps with tuber/shoot ratio was 0.269. High temperature caused a difference on growth proportions on plant part dry weight from 30 to 85 dap between high and medium land.

The ratio of each plant part towards those total dry weight was higher in high land than in medium land. That ratio showed the dynamic of photosynthate translocation and determined the differences of photosynthate distribution ability among varieties which resulted a significant differences of tuber production between high and medium land.

Keywords: Potato, dynamic growth, photosynthate translocation, medium land, Indonesia

Introduction

In Indonesia, potato plants are cultivated on the high lands (1000-2500 meter above sea level/m asl) with average air temperatures are between15-25 °C. In order to increase the potato's productivity, the land extensification is needed. On the other hand, the extensification will affecting the highland sustainability. Therefore, it is necessary to expand potato cultivation area to the lower land or called as medium land (300-700 m asl). However, the air temperature in medium lands are higher than in the highlands and become major obstacle on the potato's cultivation. [1]

Reported that the unmanageable and most important factor affecting potato growth and yield is temperature. The previous studies [2], [3], [4] had proven that potato plants were very sensitive with the increasing of the air temperature and followed by increasing soil temperature. High temperatures could drastically reduce potato tuber production [5]. High soil temperature, will result on imbalance ratio between source and sink and delaying tuber initiation and development [6].

Almost all potato varieties which have been introduced in Indonesia are suitable to grown in high land region with low temperature condition. Eight varieties used in this study were introduced for high land region, those were ‘Agria’, ‘Andina’, ‘Amabile’, ‘Cingkariang’, ‘Granola L’, ‘Granola K’, ‘Margahayu’, ‘Olympus’ and ‘Tejo MZ’. [7], [8]. Until recently, no information was available to explain the photosynthesis responses of 9 potatoes varieties when planted in medium land with high air temperature. Therefore, this is the first research to determine the changing of growth and photosynthate translocation of 9 potatoes varieties in high and medium land.

Research Methods

Research design

This research was designed as factorial experiment with two factors. The first factor was location with difference altitude and the second factor was varieties. The first location was at 1360 m asl (highland) and the second location was at 380 m asl (medium land). We use nine varieties adaptive to the highland cultivation (‘Agria’, ‘Andina’, ‘Amabile’, ‘Cingkariang’, ‘Granola L’, ‘Granola K’, ‘Margahayu’, ‘Olympus’ and ‘Tejo MZ’). For the environmental design, a randomized completely block design was used. The field experiment was conducted with 3 blocks as replications, 30 plants each variety. For the seed, a tuber each about 20-40 grams with 2-5mm sprout length were planted in the andosol soil from the highland’s region. The soil was filling in the polybag (30x50 cm) and each polybag with 30x75 cm spacing. Plant maintenance was carried out based on medium land potato cultivation technology from IVEGRI [9] whit slight modification according to the conditions during the study.

Observation

The observation of soil temperature parameters was carried out using Hobo data logger for soil temperature type TidbiT® v2 Temp (UTBI-001), which installed in the soil with 10-15 cm depth during planting season. Observation of the air temperature parameters for high land was recorded using Hobo data logger type U14-002 with sensor type S-THB-M008 which installed read the air temperature every hour during 24 hours continuously. The data of maximum and minimum daily air temperature for medium land were obtained from BMKG in Semarang city, Central Java.

Observation of fresh tuber weight per plant was carried out when the crop conditions were ready for harvest, which 50% of the potato leaves had yellowed. The harvest time in the highlands was at 89 day after planting (DAP) and in the medium land was at 95 DAP. The average data of tubers fresh weight per plant were generated from five plants in each experiment unit. The photosynthate translocation from shoot to tuber could be approached by the ratio between tuber dry weight toward total plant dry weight which consisted of shoot, root and stolon at 60 DAP. The delta ratio of plant parts toward total plant dry weight from 30 to 85 DAP was calculated from the summation of all dried plant parts.

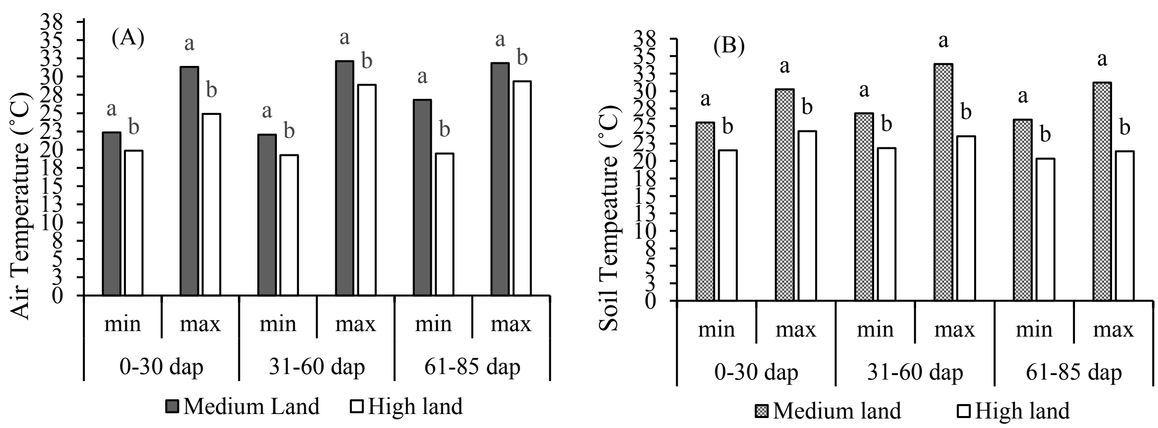
Data analysis

The data in each location were analyzed by analysis of variance using a general linear model with the SAS program. Furthermore, for significantly different results were separated using the Duncan Multiple Range Test at a significant level of 5%.

Results and Discussion

Minimum Maximum Air and Soil Temperature in the High and Medium lands at 0-30, 31-60 and 61-85 day after planting

Soil temperature tend to be higher than air temperature both in high and medium land, especially minimum soil temperature which occurred at the night to morning. It suggests that high temperature in the day would keep in the soil much longer and affecting the soil temperature at night time. Hinger temperature in the soil will reduce tuber yield by between 65-96% [1].



Note: Graph followed by different letter are significant different based on Duncan's multiple range test on 5% level

Fig. 1. Minimum and Maximum Air Temperature (A) and Soil Temperature (B) in High and Medium Land during One Planting Season

[10] Reported that the optimum air temperature for the vegetative growth phase were 20-25 °C and 15-22 °C when the tuber formation phase begins. The average air temperature in the medium land during this study were exceeded the optimum temperature for vegetative and tuber formation phase This condition indicate as the main factor which affected the potato yield in medium land.

Fresh tuber weight of nine varieties in the high and medium lands

The fresh tuber weight of all nine varieties in the high land were significantly higher than the potato tuber from medium land with the level of the declining was different among varieties (Table 1). Only four of nine varieties which cultivated in the medium land produced more than 100 grams fresh tubers weight per plant which were 'Andina', 'Cingkariang', 'Margahayu' and 'Olympus' (Table 1). It indicated that just four varieties tend to have better adaptation to survive in the high temperature condition in medium land. Therefore, further discussion in this paper only focus on those 4 varieties.

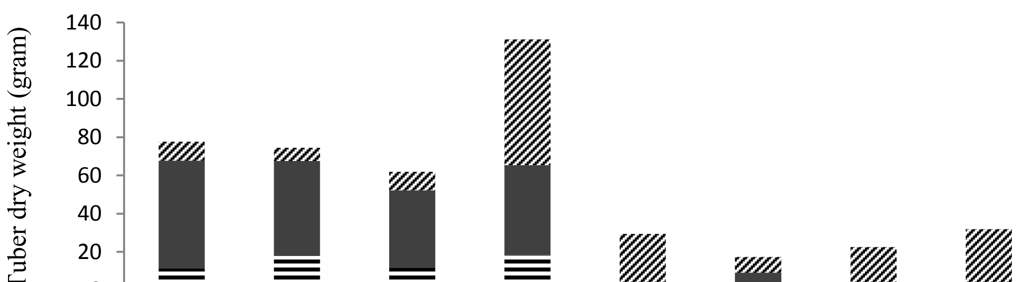
Table 1. Fresh tubers weight per plant and percentage reduction of 9 varieties in the high and medium land

Varieties	High Land (g/plant)	Medium Land (g/plant)	Percentage of yield reduction (%)
‘Olympus’	874.89 a	212.2 e	75.7
‘Amabile’	766.38 a	82.5 ef	89.2
‘Agria’	618.93 b	98.71 ef	84.1
‘Granola K’	583.08 bc	47.5 f	91.9
‘Andina’	517.42 bcd	195.5 e	62.2
‘Tedjo MZ’	510.47 bcd	39.9 f	92.2
‘Cingkariang’	496.05 bcd	115.2 ef	76.8
’Granola L’	475.35 cd	14.6 f	96.9
‘Margahayu’	412.12 d	150.6 ef	63.5
(+)			

Note: (+) Interaction between location and varieties are significant at P<0.05. Value followed by different letter are significant different based on Duncan’s multiple range test on 5% level

Tuber dry weight of 4 varieties in the high and medium lands

During the last stage vegetative growth phase (30 days after planting) all varieties have formed tubers at the highland cultivation. In contrary, tubers formation was only shown by ‘Andina’ and ‘Cingkariang’ at the medium land cultivation (Fig. 2). In the high land cultivation, during the tuber bulking phase (61 days – harvest time), accumulation of tubers dry weight was start to decrease because most of the photosynthate had been translocated to the tubers and photosynthesis process had decrease already as the leaves were yellowing (Fig. 2). In line with [11] which concluded that in the normal temperature conditions, the dry matter during vegetative growth phase was mostly translocated to the leaves and stems with a proportion of 80%: 20%. Conversely, in medium land the dry weight accumulation started during tuber bulking phase. It showed that in the medium land, the period of tuber growth was shorter than in high land. In line with [12], [13] which implied that abnormal proportion of photosynthate distribution will occur in the varieties which sensitive to high temperature. ‘Olympus’ showed a tendency of higher tuber dry weight accumulation than others varieties from 61 days – harvest time (Fig 2).

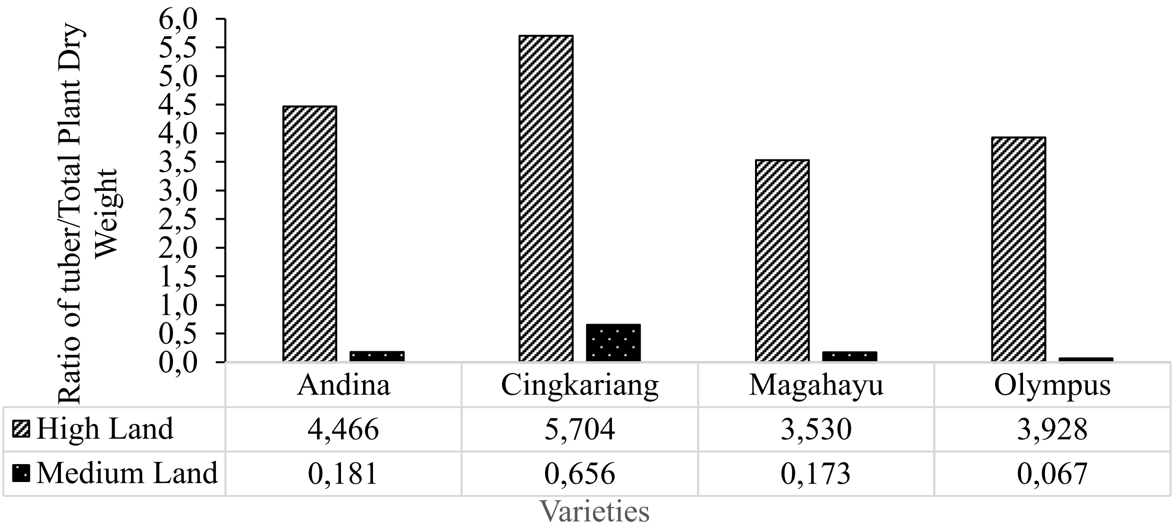


	Andina	Cingkariang	Margahayu	Olympus	Andina	Cingkariang	Margahayu	Olympus
	High land				Medium land			
61 dap-harvesting	9,91	6,79	9,60	65,93	28,02	8,22	21,50	30,88
31-60 dap	56,44	49,84	40,48	46,87	1,24	8,44	1,09	0,96
0-30 dap	11,26	17,77	11,73	18,44	0,06	0,62	0,00	0,00

Fig. 2. Accumulation of tuber dry weights of 4 varieties in three growth phases in the high and medium Land

Photosynthate Translocation of 4 Potato Varieties in the High and Medium lands at 60 days after planting

The ratio between tuber dry weights toward total plant dry weight of all varieties in the medium land at 60 days was lower than that in the high land (Fig. 3). It showed that in the high land, potato plants were succeeding in preparing sinks organ earlier since having sufficient deposit photosynthate in the shoot to be translocated to the sink (tubers). In the medium land, at 60 days after planting the potato plant was not have sufficient amount of photosynthate that can be translocated to the tuber. The ratio between tuber dry weight toward total plant dry weight also shows the readiness of the sink organ to receive photosynthate from the source (shoot). It means that the higher the ratio, the greater the amount of photosynthate which could receive by the sink.



	Andina	Cingkariang	Magahayu	Olympus
High Land	4,466	5,704	3,530	3,928
Medium Land	0,181	0,656	0,173	0,067

Fig. 3. Ratio of tuber toward total plant dry weight at 60 DAP in the high and medium land

Delta plant part dry weight accumulation toward total plant dry weight from 30 days to 85 days of 4 varieties in the high and medium lands

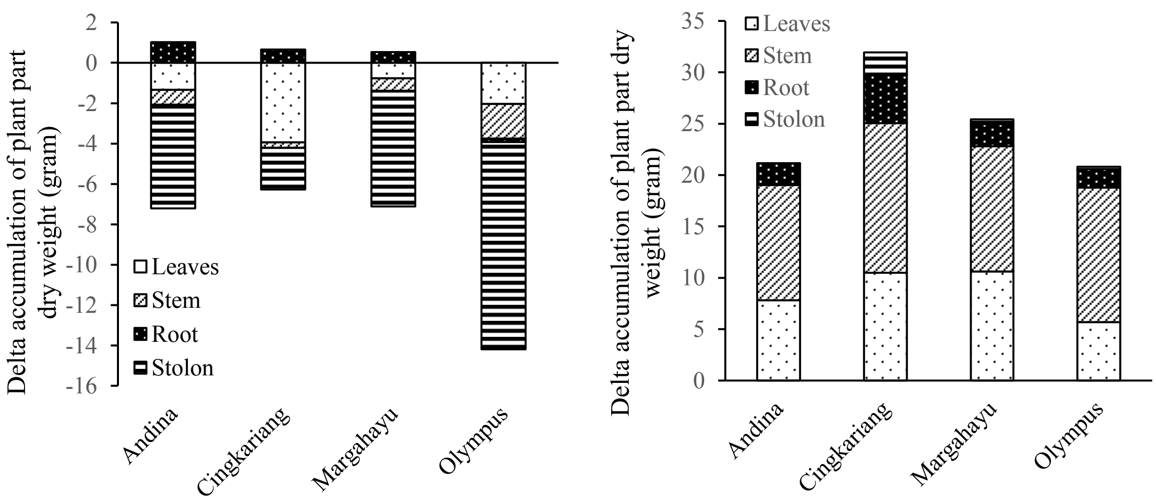


Fig. 4. Delta plant part dry weight accumulation toward total plant dry weight of 4 varieties from 30 to 85 DAP in high land (a) and medium land (b)

There was a difference between that delta plant part dry weight accumulation which occurred in potato plants in the medium land and high land. In the high land, the delta plant part dry weight accumulation toward total plant dry weight was shown a negative value except roots (Fig. 5.a).

Those results in in opposite with the medium land, which still continued to increase along with the plant age (Fig. 5.b). Prolongation of vegetative phase in the medium land would explain why the potato production was very low. These condition in line with [2] who stated that the decreasing in tuber production was not only caused by the rate of photosynthate distribution to the tubers but also because of the reduced on tuber filling time due to the lengthening of the vegetative phase.

Tendency of lower delta plant part dry weight accumulation toward total plant dry weight showed by ‘Olympus’, which explained that this variety has a better potency to suppress its vegetative growth than other varieties during tuber bulking phase in medium land.

Conclusion

The ‘Olympus’ variety was able to increase vegetative growth from 30-60 DAP and able to suppress vegetative growth during tuber bulking phase. It shows that the amount of photosynthate distributed to tubers were higher and produce higher tuber fresh weight than others varieties in medium land.

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Selection and Evaluation of Five Chili Mutant Genotypes for Resistance to ChiVMV and Yield in Lembang, West Java

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Abstract

Chili Veinal Mottle Virus (ChiVMV) has become one of important causes of chili yield decrease in Indonesia. The objectives of this study are to examine the resistance level of five M_6 chili genotypes to ChiVMV infection and to test their performance in terms of yield quantity and quality. The study was conducted at Indonesian Vegetable Research Institute (IVEGRI)-Lembang (1,250 m above sea level) from April to December 2016. The five M_6 chili genotypes evaluated are M_6. 353.1; M_6.238.2; M_6.801.1; M_6.517.2; M_6. 113.3 with three reference varieties i.e., Gelora, Ciko, and Tanjung-2. Randomized block design was used with four replications. Results from artificial inoculation at screen house show that genotypes M_6.353.1, M_6.517.2, and Tanjung-2 are resistant against ChiVMV with disease intensity percentage less than 10%. The yield weights of genotypes M_6.517.2 (32.77 kg/10.5 m^2) and M_6.113.3 (34.17 kg/10.5 m^2) are not significantly with Ciko (36.74 kg/10.5 m^2) as a reference variety. Some of M_6 chili genotypes even perform better than the three reference varieties in terms of skin thickness, storability, vitamin C and capsaicin levels. Genotype $M_6$517.2 is proposed as a candidate for new variety since it is resistant to ChiVMV and also it has good quality and quantity of yield.

Keywords: Chili, Chili Veinal Mootle Vir*us, mutant, quality and quantity of yield*

Introduction

The highest national chili productivity in Indonesia was West Java of 15.00 t/ha [1]), but it was still lower than chili productivity of the world (16.11 t/ha) [2]. *Chilli Veinal Mottle Virus* (ChiVMV) is one of the important pathogens commonly found in chili plants in Indonesia [3]).

ChiVMV infection could reduce the quality and 60–100% of yield losses of chili [4]). In Indonesia the existence of ChiVMV had spreader widely in West Java, Central Java, East Java, South Kalimantan, West Sumatra, Central Aceh, and South Sulawesi [5, 6].

The resistant chili variety is needed as one of the components in Integrated Disease Management (IDM) [7]. The results of research collaboration between IVEGRI and ICABIOGRAD (2015-2016) had succeeded in obtaining five M_6 chili genotypes derived from *in vitro* mutation by mutagene *Ethyl Methyl Sulfonate* (EMS) that resistance to ChiVMV. However, consumer’s preference of the new chili variety not only for disease resistance, but also for yield quality and quantity. The diameter, length, weight, color, skin smoothness, and shelf life as well as the pungency level and vitamin content are some of the chili criteria could be accepted by consumers [8]). The objectives of this study are to examine the resistance level of five M_6 chili genotypes to ChiVMV infection and to know their performance in terms of yield quantity and quality.

Materials and Methods

The experiment was conducted at Indonesian Vegetable Research Institute (IVEGRI), Lembang (1,250 m asl) from April to December 2016. This experiment used randomized block design with four replications (30 plants/plot/replication, spacing of 50x70 cm). The five chili M_6 genotypes evaluated were M_6. 353.1, M_6.238.2; M_6.801.1; M_6.517.2, M_6. 113.3 with three reference varieties i.e., Gelora, Ciko, and Tanjung-2.

A resistance screening test to ChiVMV infection was conducted at the screen house as additional data if intensity of pathogens in open field is less. The Inoculum of Cikabayan isolate was used because the most virulent in Indonesia [6]). Mechanically artificial inoculation was done onto leaf surface of seedlings with carborundum (600 mesh) by cotton buds. The screened number were 10 plants per each genotype without replication.

The symptoms of ChiVMV infection were evaluated with scoring 0-6 [9]): (1) Scoring 0: no symptoms; (2) Scoring 1: mild stripes; (3) Scoring 2: moderate stripes, no malformation; (4) Scoring 3: moderate stripes, malformation; (5) scoring 4: severe stripes, malformation; (6) scoring 5: severe stripes, malformation, shoestring; (7) scoring 6: severe stripes, malformation, shoestring, dwarf. Diases intensity is calculated by the formula [10, 11]: $IP= (\Sigma \text{ (v x n)}/Z \text{ x } N) \text{ x } 100\%$.

Quantitative data was analysed to use variance analysis and be continued by Duncan's Multiple Range Test as an advanced test.

Results and Discussions

Naturally Disease Infection in Open Field and Artificial Inoculation at Screening House

It can be seen that the percentage of disease incidence of ChiVMV in open field is very low on each genotype (0-1.19%), eventhough the inoculum is sufficient by spreading the ChiVMV infected plant artificially (Table 1). Naturally, ChiVMV is transmitted by several insect vector such as *Myzus persicae*, *Aphis gossypii*, *Aphis craccivora*, *Aphis spiraecola*, and *Hysteroneura setaria*. The virus transmission is done non-persistently by the insect vector. They get the virus by sucking infected plants just a few seconds, then they will transmit the virus quickly on healthy plants. After that the insect vectors will lose the virus and they cannot afford to transmit the virus to other plants [12, 13]. Some factors contribute in transmitting the virus and one of them is vector insect number and their mobility. According to observed data (30-115 DAP) shows that average number of ChiVMV vector insects are not abundant and mostly found in the genotype M_6.801.1 (4 insect vectors/plant) while others genotypes in range 2-3 insect vectors/plant. This situation is predicted as a causal factor from low ChiVMV transmission in open field until disease incidence percentage is low also.

Another causal factor is latent infection or masking occurrence, so that the symptoms doesn't perform. In this case a plant which has no disease symptom is not means as a healthy plant.

Environmental factors i.e., temperature, humidity, rainfall is very influential on occurrence disease symptom [6]. In the fact, the disease symptom doesn't perform at condition low temperature and high rainfall intensity. On the other hand, ChiVMV might also infect chili plants together with another virus (e.g., CMV) that caused biased symptom [13, 14]. Murphy and Bowen [15] stated that when two type viruses infected plant together, the symptoms could cause by a single virus only and another didn't perform. Generally, the symptom of a mixed infection of two type virus was more severe and they were called synergistic [16]. In the Table 1 can, be seen that genotypes M_6.353.1, M_6.517.2, and Tanjung-2 are resistant to ChiVMV because their disease intensity is below 10%.

Table 1. Disease incident and intensity of ChiVMV infection as an inoculation result by naturally in open field and artificially at the screening house

Genotypes	Open field (30-115 DAP*)		Screening house (80 DAI*)		
	Disease incident (%)	Insect vector number per plant (unit)	Disease incident (%)	Disease intensity (%)	Resistance category**
Gelora (M0)	0.60	3	14.07	14.41	Rather resistant
M_6.238.2	0.00	3	46.67	45.56	Susceptible
M_6.353.1	0.00	2	9.69	8.59	Resistant
Tanjung-2	0.60	2	13.33	3.33	Resistant
Ciko	0.63	2	26.67	21.11	Rather susceptible
M_6.517.2	0.60	3	7.04	7.04	Resistant
M_6.801.1	1.19	4	33.33	31.11	Susceptible
M_6.113.3	0.60	3	37.78	37.77	Susceptible

**DAP: Days after planting; DAI: Days after inoculation*

***Very resistant (VR) = 0.00%; Resistant (R) ≤10%; Rather resistant (RR): 10%<DI≤20%; Rather susceptible (RS): 20%<DI≤30%; Susceptible (S): 30%<DI≤50%; Very susceptible (VS): DI>50%.*

Table 2. Plant morphological size of five chili mutant M_6 and three reference varieties in Lembang (115 DAP)

Genotypes	Plant height (cm)	Plant canopy (cm)	Stem diameter (mm)	Leaf lenght (cm)	Leaf diameter (cm)	Flowering time (DAS)	Harvesting time (DAS)
Gelora	56.33 b	44.91 bcd	12.23 b	6.92 b	2.92 bc	54.50 a	91.88 a
M_6.238.2	63.75 ab	43.41 d	13.61 ab	6.61 b	2.73 c	51.00 b	92.63 a
M_6.353.1	60.25 ab	44.04 cd	12.51 b	6.87 b	2.85 bc	52.00 ab	91.9 a
Tanjung-2	45.30 c	52.31 a	14.71 a	7.28 b	3.12 ab	45.00 c	71.50 b
Ciko	50.58 c	51.33 ab	14.29 a	8.44 a	3.42 a	50.50 b	73.88 b
M_6.517.2	64.00 ab	47.73 abcd	13.52 ab	6.65 b	2.70 c	51.25 b	91.79 a
M_6.801.1	60.33 ab	41.92 d	12.24 b	6.42 b	2.80 c	54.25 a	92.00 a
M_6.113.3	66.10 a	50.61 abc	13.85 ab	7.09b	2.94 bc	51.75 ab	91.71 a
CV (%)	8.12	9.05	7.50	7.30	6.09	3.39	2.02

Mean followed by the same letters on the same columns are not significantly according to Duncan's multiply range test at 0.05 level; CV: coefficient variance

Plant Growth and Development in the Field

In the Table 2 can be seen that genotype M_6.113.3 has the highest and largest plant size based on plant height, canopy widht, and stem diameter. Plant growing type of all chili mutant genotypes are erect, similar with Gelora as their parent type. The advantage of this type is more denser in spacing, so plant population number per area can be much more also. On the contrary, genotype Tanjung-2 (52.31 cm) and Ciko (51.33 cm) are wider for plant canopy size because they have prostate and intermediate types. Genotype Tanjung-2 (14.71 mm) and Ciko (14.29 mm) have larger size than others for stem diameter eventhough they both are not different significantly with genotype M_6.517.2 (13.52 mm), M_6.238.2 (13.61 mm), and M_6.113.3 (13.85 mm). Five chili mutant and Gelora as their parent type have lanceolate type for leaf's morphology. This fact caused their leaf size are smaller than genotypes Ciko and Tanjung-2 which have leaf in ovarian type.

This result is in accordance with Gunaeni *et al.*, [17] that mentioned the leaf shape of these five-mutant chili M_6 were same with Gelora as their parent type. Genotypes Tanjung-2 and Ciko also are earlier in flowering and harvesting time because they have determinate type, while all mutant genotypes and Gelora are indeterminate type.

Yield quantity and quality of five chili mutant M_6 in Lembang

Fruit length and fruit diameter are a character determinant of chili ideotype to be accepted by consumers [8, 18]. It is mentioned that the chili fruit diameter of consumer's preference in ranges from 1.0-1.5 cm and fruit length between 10-13 cm. According to these criteria, the fruit ideotype of all mutant genotypes fulfill requirements to be accepted by consumers. The fruit shape tends to be straight and slimmer compared to Gelora as parent type. It can be seen also that statistically genotype M_6.517.2 (32,767.80 g) and M_6.113.3 (34,171.13 g) have fruit weight per plot (10.5 m^2) are not different significantly with genotype Ciko (36.735.30 g) as the heaviest (Table 3).

Table 3. Yield quantity of five chili mutant M_6 and three reference varieties in Lembang

Genotypes	Fruit lenght (cm)	Fruit diameter (mm)	Fruit number per plant (unit)	Fruit number per plot (10.5 m^2)	Fruit weight per plant (g)	Fruit weight per plot (kg/10.5 m^2)
Gelora	12.19 b	15.39 b	149.50 a	4358 a	1047.20 b	30.47 bcd
M_6.238.2	12.28 b	14.64 bc	132.75 ab	3943 ab	988.50 b	28.07 d
M_6.353.1	12.08 b	14.93 bc	121.75 ab	3579 ab	968.25 bc	27.30 d
Tanjung-2	10.57 c	18.56 a	94.50 b	2797 b	738.45 c	20.53 e
Ciko	13.74 a	17.28 a	97.25 b	4371 a	1249.50 a	36.74 a
M_6.517.2	12.24 b	14.20 bc	155.00 a	4433 a	1096.50 ab	32.77 abc
M_6.801.1	12.35 b	14.82 bc	143.75 a	4068 a	993.25 b	28.51 cd
M_6.113.3	12.04 b	13.54 c	156.75 a	4470 a	1166.25 a	34.17 ab
CV (%)	3.09	7.00	19.18	19.20	8.97	8.90

Mean followed by the same letters on the same columns are not significantly according to Duncan's multiply range test at 0.05 level; CV: coefficient variance

Table 4. Yield quality of five chili mutant M_6 and three reference varieties in Lembang

Genotypes	Fruit skin thickness (mm)	Shelf life (DAS)	Vitamin C (mg/100g)	Capsaicin level (ppm)
Gelora	2.40 e	13.09 b	113.71 e	400.25
M_6.238.2	2.68 cd	13.83 ab	145.47 c	555.62
M_6.353.1	2.76 cd	14.00 a	126.07 d	305.38
Tanjung-2	2.28 e	8.25 c	84.06 f	335.12
Ciko	2.17 e	8.17 c	158.16 b	345.72
M_6.517.2	3.26 ab	14.17 a	108.77 e	2600.96
M_6.801.1	2.82 bc	14.04 a	170.62 a	1661.46
M_6.113.3	3.38 a	14.50 a	133.59 d	2421.12
CV (%)	5.36	3.70	4.60	

Mean followed by the same letters on the same columns are not significantly according to Duncan's multiply range test at 0.05 level; CV: coefficient variance

Skin thickness is one of the requirements consumer's preferences because correlated with ease of processing in cooking or shelf life. Consumers prefer skin thickness in the range 3.0 - 6.0 mm [8]. Thus, genotypes M_6.113.3 (3.38 mm) and M_6.517.2 (3.26 mm) are qualify in preference's consumer. However, mostly five mutant chilies have shelf life are longer than three references variety (Table 4). The average vitamin C content of four M_6 chili mutant in ranged from 126.07 to 170.62 mg/100 g. The capsaicin testing using HPLC method shows that three M_6 chili mutant having capsaicin level above 1000 ppm, i.e., M_6.517.2 (2600.96 ppm), M_6.801.1 (1661.46 ppm), and M_6.113.3 (2421. 12 ppm).

Adiyoga and Nurmalinda [19] stated that consumers prefer chili that has the characteristics of bright-red skin color, big-hot peppers type, and slightly hot. Within the context of measuring preference, the most important chili attribute that influences consumer decision making were skin color, and then followed by chili type, and hotness. Furthermore, the size of chili diameter that preferred by consumer ranges from 1.0 to 1.5 cm with skin thickness is around 0.3-0.6 cm, fruit length around 10-12 cm, and pungency level more than 300 ppm [8, 20]. According to multi character selection can be known that genotype M_6. 517.2 can be proposed as a new variety candidate that resistant to ChiVMV and suitable for consumer's preference.

Conclusion

Genotype M_6.353.1, M_6.517.2, and Tanjung-2 are resistant against ChiVMV with disease intensity percentage less than 10%. The yield weights of genotypes M_6.517.2 (32.77 kg/10.5 m^2) and M_6.113.3 (34.17 kg/10.5 m^2) are not different significantly with Ciko (36.74 kg/10.5 m^2) as reference variety for yield. Some of mutant chili M_6 even perform better than the three reference varieties in terms of skin thickness, storability, vitamin C, and capsaicin level. Genotype M_6.517.2 is proposed as a candidate for new variety since it is resistant to ChiVMV and has good quality and quantity of yield.

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Developing Drought Tolerant Potato Through Conventional Breeding Towards Food Sufficiency: Selection on the Segregation Population

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Abstract

Drought stress becomes the most environment limiting factor in crop production. Recently, drought condition increases either in intensity or distribution. Due to the shallow and small root architecture, potato is sensitive to water deficit. To keep the tuber yield, we need potato cultivars with tolerance to drought. Conventional breeding by manual crossing followed by progeny selection have been done in 2014 and 2015, to get the superior progeny under water deficit condition. These activities were carried out in the green house of Indonesian Vegetable Research Institute (IVegRI) located at Lembang, West Java (altitude 1250 m). Nine hundred seedlings from 18 segregation population used for selection. Watering was given in interval 3 days in field capacity. Water treatment was performed on the 30 days old plants by extending the interval of watering, from 3 days to 7 days. Water stress treatment left 36.22% plants at the end of treatment.

The segregation population from the 5.13 x cv. Atlantik gave the most surviving plants. Sixty individual plants were selected from the survived plants based on tuber yield. The selected genotypes then become breeding line materials for the next selection and evaluation, in order to get the drought tolerant potato cultivars.

Keywords: Climate change, food sufficiency, potato breeding, tuber yield, water deficit

Introduction

The majority of earth's population relay their food on Gramineae family crops, such as sorghum, wheat, and rice. Nowadays, potato, a member of Solanaceae family, become the important food crop in the world, number three after wheat and rice. This crop had harvesting area exceed from 19 million hectares, produced more than 381 million ton in 2014 and consumed over than 239 million ton (FAO 2017). Moreover, potato tubers not only used for fresh food but also for raw material in some industries, such as processed food, flour, starch and ethanol [1], [2].

Became the most important main food in developed countries, now potato production and consumption increase significantly in the developing countries. This phenomenon was driven by some facts, such as wide adaptive range of environment, easy cultivation and high nutritional value [3].

Climate change has become the main issue in agricultural sector, as food crops are highly depending on their environment. Global potato yield was predicted decrease 18 to 32% due to climate change by 2050 [4]. One of the effects of climate change is drought. In agriculture sector, drought is a condition when the availability of soil moisture to plant has dropped to a level that affects the crop yield adversely [5]. Even though potato is known as a productive water use crop [6], this crop is sensitive to drought stress [7]. This sensitivity due to its shallow root architecture, and short and small size of stolon roots [8]. Under field condition, drought stress caused significant tuber yield reduction, till 54% [9], [10]. This reduction as a result of impairment of many physiological and biochemical processes of plant during drought stress, such as photosynthetic rate reduction [11] and less sucrose transported to tuber [12]. Tuber formation is the most sensitive phase to the drought stress compared to another developmental phase [13].

Since climate change with its environmental consequences, includes drought, is inevitable, we need to concern to our food sufficiency in some next decades. We can alleviate the potato yield loss under drought condition by cultivation management, such as using mulch [14] and irrigation schedule [15]. The other way is by using drought tolerant potato cultivars. By such adaptations, the reduction of global potato yield can be suppressed become 9 to 18% [4]. We conducted conventional breeding to develop potato cultivars adapted to climate change effect, include drought stress. Crossing used some parents with various background have been done in IVegRI.

This research aimed to select the drought tolerant genotypes from segregation population derived from crossing in the green house condition.

Methodology

The study was carried out in the green house at IVegRI, Lembang, West Java, Indonesia (altitude 1250 m). There were 18 segregation population derived from 11 parents used in this study. All parents are breeding lines, except cultivar ‘Atlantik’. The manual hybridization was done in 2014, were as selection activity from April to August 2015. The seeds were sown in the plastic tray filled with sterilized media (charcoal husk: manure: soil = 2:1:2). Seedling with 3-4 true leaves were transplanted into 5L polybag contained of sterilized media (charcoal husk: manure: soil = 1:4:2) and NPK fertilizer (2 g per polybag). One population segregation derived from one crossing consisted of 50 seedlings. Watering to field capacity was done in interval 3 days with 500 ml of water each polybag. Selection pressure was given 30 days after planting (DAP), by extending the interval of watering, from 3 days to 7 days. This means the plant received only 43 % of watering from previous.

Plant height at 0 day after treatment (DAT), 15 DAT, 30 DAT; number of survived plants at 0 DAT, 15 DAT, 30 DAT and 60 DAT (harvesting), number of tubers per plant and tuber characters were observed and recorded.

Results and Discussion

For the selection, we chose the normal and healthy seedling, which characterized by good vigor, green leaves, normal leaves (not curly), and did not show any disease or pest attack symptoms.

Before water treatment, plants grown well, with the variation of growing rate among them, this is due to genetic variation as a consequence of sexual hybridization of potato. The cultivated potato is highly heterozygous and consequently, there are highly segregation of F1 generation resulted from hybridization [16].

Descriptive analysis in plant height revealed that generally the variation of plant height increased as plant grown older (Table 1). Populations of CIP 394613.139 x 5.13, 3.11 x AKRb.134 (a) and Atlantik x 1.4 had slower growth than other population after received water treatment, i.e., 83.55%, 93.25% and 99.11% respectively. Whereas population 2.13 x Atlantik (a) had highest growing rate in 30 days after treatment, i.e., 186.63%. Plant height is one of morphological parameter that negatively affected by drought stress and the effect more severe when drought come in early stage [17].

Table 1. Descriptive analysis of plant height

Progeny	0 dat					15 dat					30 dat				
	Min	Max	SD	Ave	CV	Min	Max	SD	Ave	CV	Min	Max	SD	Ave	CV
2.13 x AKRb.134	13	41	5.82	23.60	24.66	14	63	12.17	32.15	37.85	21	101	27.40	54.68	50.11
2.13 x GKRb.204	6	34	5.50	21.65	25.38	16	56	8.83	30.54	28.90	27	105	20.53	49.69	41.32
2.13 x Atlantik (a)	12	32	4.79	21.17	22.63	20	65	10.89	32.07	33.94	29	115	27.10	60.68	44.65
5.13 x Atlantik	12	30	4.86	20.64	23.57	19	70.5	11.58	36.05	32.11	25	120	22.16	56.97	38.89
AKRb.134 x 7.A	12	32	4.69	21.65	21.68	21	87	12.62	34.21	36.89	29	110	20.88	48.42	43.13
CIP 394613.139 x 7.A	7	34	4.98	21.58	23.08	10	87	14.75	34.51	42.73	20	125	23.74	47.61	49.86
2.13 x Atlantik (b)	14	39	5.47	24.08	22.71	19	77.5	11.97	35.77	33.46	25	110	22.11	50.98	43.37
CIP 394613.139 x 2.7	7	31	4.59	16.74	27.42	10	77	11.60	29.81	38.90	25	85	13.72	42.57	32.23
Atlantik x 3.11	11	32	5.28	20.29	26.01	18	51	8.26	30.50	27.08	25	68	15.24	45.76	33.30
CIP 394613.139 x 5.13	12	34	5.14	21.95	23.42	17.5	76	13.72	35.87	38.26	25	71	15.41	40.29	38.26
3.11 x AKRb.134 (a)	14	38	5.15	24.16	21.32	10	63	10.93	36.51	29.95	17	65	13.57	46.69	29.06
Atlantik x 1.4	16	37	5.26	24.66	21.33	22	66.5	10.93	37.76	28.94	29	73	14.63	49.10	29.80
9.3 x Atlantik	9	32	6.96	20.14	34.55	7	74.5	13.04	31.66	41.18	15	92	23.37	47.15	49.55
3.11 x AKRb.134 (b)	5	35	8.08	17.70	45.66	15	54	10.02	31.69	31.61	25	76	14.78	46.23	31.98
3.11 x Atlantik (a)	5	32.5	7.21	21.45	33.64	20	79	11.42	37.42	30.51	35	75	12.47	49.80	25.05
CIP 394613.139 x 1.4	5	45.5	9.44	22.78	41.45	17	61	10.98	36.56	30.04	29	82	14.57	60.13	24.23
3.11 x Atlantik (b)	7	39	8.08	19.84	40.73	12	70	12.99	33.63	38.61	20	118	26.77	51.81	51.66
5.13 x GKRb.204	7	39	8.89	22.58	39.36	14	70	11.76	37.31	31.51	25	121	25.75	53.00	48.58

dat: days after treatment; Min: Minimum; Max: Maximum; SD: Standard Deviation; Ave: Average; CV: Coefficient of Variation

Total population used in this selection program were 900 plants. Up to 45 days or 15 days after watering reduction, the number of survived plants was 100%, although some individuals experienced drought and showed wilt symptom, but they refreshed after watering. Five percent of plants collapsed at one month after drought treatment (60 days old plants), and there were 853 individuals surviving with wilted or fresh condition. Furthermore, drought conditions obtained through watering reduction, left 326 individual plants (36.22%) only, at the age of plant 90 days.

The rest survived plants showed symptoms of drought stress such as withering with stems and leaves remaining green, withered with yellowing leaves, or fresh with yellowing leaves. The dried plants in both leaves and stems were categorized as dead plants. At the harvest time (90 dap), progeny CIP 394613.139 x 5.13 had the fewest survived plants (5 plants), while the 5.13 x Atlantic had the most survived plants (29 plants).

Based on descriptive analysis, generally, the variation in tuber weight per plant was not highly different with variation of tuber number (Table 2). Population 5.13 x GKRb.204 had the highest maximum tuber number (27 tubers) and variation of it (85.32%). Whereas for tuber weight per plant, 5.13 x Atlantik had the highest maximum value (167 g) and 2.13 x GKRb.204 had the highest variance 88.41%. The good tuber production under water deficit condition is one of the highly required characteristics, since the genotype can maintain yield and produce an economic yield [18], [19].

Although at harvest time there were 326 individual plants (36%) that survived, not all of them were selected. Selection was conducted based on tuber production and the uniformity of tuber size per plant. Although showing tolerant to drought during vegetative stage, some progenies had no selected individuals because of two reasons, i.e. they have very low tuber production with a small tuber size or they produced many tubers but did not uniform in size. These progenies include CIP 394613.139 x5.13, Atlantik x 1.4, 9.3 x Atlantik, 3.11 x AKRb.134 (b), and CIP 394613.139 x 1.4. Drought in plant breeding programs is more associated with inadequate supply of water as a source of moisture, causing a decrease in yield [20]. Therefore, selection is not only carried out based on the ability of plants to survive in drought conditions, but rather emphasizes on its production capacity. It was mentioned that in potato, drought-tolerant genotypes have higher production capacity than drought-sensitive genotypes [21]. Without the use of tolerant variety, drought stress causes production decline from 11 to 53% [10].

Considered the tuber production and uniformity of tuber size, in this study we obtained 60 individual plants, or 6.67% of the initial population (Table 3). The tuber number of selected individuals ranges from 3 to 25 tubers per plant. While the tuber weight per plant ranges between 32 and 167 g. The condition of survived selected individuals at harvest time varied, i.e., wilting (13.33%), senescence (43.33%) and stay green (43.33%). Stay green is an important trait to keep plant yield under water restriction condition, due to biomass accumulation under water stress [22].

Table 2. Descriptive analysis of tuber yield

Progeny	Tuber number					Tuber weight (g)				
	Min	Max	SD	Ave	CV	Min	Max	SD	Ave	CV
2.13 x AKRb.134	1	23	4.19	6.12	68.46	7	102	24.11	36.61	65.85
2.13 x GKRb.204	1	25	4.62	6.04	76.46	5	128	26.11	29.53	88.41
2.13 x Atlantik (a)	1	17	3.00	5.31	56.57	6	159	28.79	35.59	80.90
5.13 x Atlantik	1	14	2.96	5.66	52.29	2	167	30.75	34.90	88.10
AKRb.134 x 7.A	1	12	2.10	4.31	48.85	5	80	16.25	27.86	58.33
CIP 394613.139 x 7.A	2	25	4.44	5.85	75.82	6	90	19.64	30.25	64.94
2.13 x Atlantik (b)	1	13	2.87	4.98	57.73	9	69	13.97	28.90	48.32
CIP 394613.139 x 2.7	1	13	2.87	4.90	58.68	1	52	10.40	17.47	59.56
Atlantik x 3.11	1	13	2.12	3.88	54.71	1	44	8.23	16.46	50.02
CIP 394613.139 x 5.13	1	8	1.76	3.02	58.14	1	17	3.89	8.25	47.14
3.11 x AKRb.134 (a)	1	7	1.43	3.28	43.57	1	20	3.78	10.24	36.89
Atlantik x 1.4	1	8	1.75	3.56	49.20	1	28	5.32	12.10	43.99
9.3 x Atlantik	1	11	1.87	3.57	52.38	1	26	5.12	12.29	41.70
3.11 x AKRb.134 (b)	1	13	2.31	4.02	57.39	4	57	8.12	14.79	54.88
3.11 x Atlantik (a)	1	11	2.14	4.04	53.03	5	58	8.57	13.92	61.56
CIP 394613.139 x 1.4	2	9	1.62	4.10	39.57	4	29	5.74	14.14	40.59
3.11 x Atlantik (b)	1	9	1.94	4.42	43.86	4	55	11.94	17.94	66.53
5.13 x GKRb.204	1	27	4.28	5.02	85.32	3	63	13.01	17.56	74.06

Min: Minimum; Max: Maximum; SD: Standard Deviation; Ave: Average; CV: Coefficient of Variation

We use tuber number per plant, average tuber weight and tuber weight per plant in this study for the selection criteria, since these tuber yield traits were correlated positively with total tuber yield not only in optimum condition but also under drought [23]. Even though yield component (tuber number and average weight per tuber) does not suggested for indirect selection because of its low heritability [24], in this case, we used tuber yield for selection criteria for completing the surviving ability criteria under drought condition. Plant survival under drought stress condition showed the capacity of physiological tolerance, whereas tuber yield as economically part of potato that performed under drought represented agronomic tolerance [25].

For the next assessment, we propose to bring the selected genotypes to the field for seed propagation, followed by trials in two field environments, rain fed or irrigated and drought condition. In the case of potato drought tolerance breeding program, the genotypes with stable yield under both environments are more valuable to be continually breed in order to get new drought tolerant cultivar [26].

Table 3. Selected individuals for drought tolerance

Progeny	Number of selected plants	Range of tuber number per plant	Range of tuber weight (g)	Range of tuber weight per plant (g)
2.13 x AKRb 134	10	4- 18	4.94-14.57	37-102
2.13 x GKRb 204	6	6- 25	4.80-17.17	52-128
2.13 x Atlantik (a)	4	3-5	9.20-19.67	46-59
5.13 x Atlantik	12	4-14	5.00-13.73	40-167
AKRb 134 x 7.A	3	5-12	6.67-9.80	49-80
CIP 394613.139 x 7.A	8	4-10	5.90-18.00	44-90
2.13 x Atlantik (b)	7	4-17	3.18-15.90	51-159
CIP 394613.139 x 2.7	1	9	5.78	52
Atlantik x 3.11	2	4-7	6.29-8.00	32-44
3.11 x AKRb 134 (a)	1	7	8.14	57
3.11 x Atlantik (a)	1	5	11.60	58
3.11 x Atlantik (b)	2	7-9	5.67-7.86	51-55
5.13 x GKRb 204	3	4-27	2.33-14.00	56-63

Conclusion and Future Perspettive

Selection of segregation population for drought stress tolerance in the green house was conducted in simultaneous criteria, survived plants and tuber yield of survived plants. From the selection we obtained 60 individual plants or 6.67% of the initial population. The survived plants at harvest time showed stay-green character, senescence, and wilt. Tuber number from selected individuals ranged from 3 to 25 tubers per plant, while the tuber weight per plant ranged between 32 and 167 g. Next, field selection should be conducted in some cycles and multiyear, to ensure the stability of drought tolerance. From tuber yield comparation between irrigated and dry environments we expect some genotypes with stable yield under both conditions, means those genotypes has high ability to adapt and maintain the yield under various environment. Such property is needed to keep the sustainability of potato production under climate change, so that our future food sufficiency will be assured.

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Physicochemical and Organoleptic Properties of 1-Methylcyclopropene-Treated Shallot

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Abstract

Postharvest treatment using 1-Methylcyclopropene (1-MCP) has been done in many studies, in order to control respiration process of horticultural produces. While controlled respiration is of high importance in economically strategic commodities such as shallot, the main appeal of the commodity is its characteristics and organoleptic properties. The objective of this research was to study the effects of 1-MCP on the physicochemical and organoleptic characteristics of shallot. 2.5 g of 1-MCP (EthylBloc™) was used in the treatment on shallot for 0, 6, 18, 30, and 42 hours.

Physicochemical properties observed included color, texture, moisture, ash, protein, fat and carbohydrate content. The level of respondents' likeness on aroma, color, general appearance, easiness to peel and general likeness was obtained in organoleptic test. After three weeks of storage, untreated shallot had significantly lowest moisture content (dry and hollow) (75.33%) while the treated shallot maintained its moisture, even with the minimum exposure of 6 h (80.85%). Only protein content was not affected by the duration of exposure. Regarding organoleptic properties, 42h exposure of 1-MCP gave significant higher scores from the respondents, for all parameters except general appearance. It was concluded that 1-MCP exposure positively influenced the physicochemical and organoleptic properties of shallot.

Keywords: 1-methylcyclopropene, organoleptic, physicochemical properties, shallot

Introduction

1-methylcyclopropene (1-MCP) is a cyclic alkene that has been studied widely in the last decade for its ability to bind the ethylene receptor and to block ethylene action [1]. The proofs of its significant benefits in controlling ripening and senescence had been reported in numerous scientific papers, describing its effectiveness in maintaining horticultural produces quality, e.g., banana [2], tomato [3], apple [4], and durian [5]. 1-MCP was also proven more effective in inhibiting ethylene production and respiration in pack choy, compared to hydrogen sulphide [6].

However, to our knowledge, similar study on the onions group (*Allium sp*) is very limited. Shallot particularly is a very important horticultural commodity in Indonesia, being consumed daily as spice and herb for cooking in almost all areas in Indonesia. The demand is stably high throughout the year, causing extreme price fluctuation caused by postharvest losses and poor harvest yields during certain season. 1-MCP treatment may be the solution for reducing shallot postharvest losses. It was reported that 1-MCP had positive effect on onion bulb, based on the inhibited sprout growth and maintained dry weight [7]. Our previous study had also shown that 1-

MCP treatment on shallot was effective in controlling respiration rate, ethylene production rate and prevent excessive weight loss during storage [8].

While controlled respiration is of high importance in economically strategic commodities such as shallot, the main appeal of the commodity is its characteristics and organoleptic properties. The objective of this research was to study the effects of 1-MCP on the physicochemical and organoleptic characteristics of shallot.

Method

1-Methylcyclopropene treatment on shallot

The dosage of 1-MCP was determined in the previous research [8]. A commercially available 1-MCP was used (EthylBloc™) in the form of 2.5 g packaging of 1-MCP powder with the concentration of 0.014% of 1-MCP. Shallot was obtained from Brebes, Central Java. Harvest was done 1 (one) day before treatment, with the age of maturity approximately 70 days after planting.

Shallot was sorted to separate the damaged ones and cleaned from dirt and soil prior to treatment.

2.5 g of EthylBloc™ was put on top of a layer of wet-cotton in air-tight glass chambers (modification of Watkins 2006) [9]. 3 kg of shallot was left in separated chambers for 0, 6, 18, 30, and 42 hours, with controlled temperature of 21-25 ^{o}C. The experiment was carried out in two repetitions. After the designated storage duration, shallot was displayed in storage shelves with the same temperature prior to analyses.

Physicochemical analyses

Observation parameters on 1-MCP-treated-shallot included colour and texture, moisture, ash, protein, fat and carbohydrate content. Colour was measured in L, a, and b value using Chromameter and texture in hardness value using Texture Analyzer (Brookfield). Measurement on colour and texture was done 4 (four) times, i.e., initial colour (first day of display in storage shelves), 3 weeks storage, 6 weeks storage and 9 weeks storage. Indonesian National Standards (SNI 01-2891-1992) [10] was used in proximate analyses including moisture (every 3 days), ash, protein, fat and carbohydrate content (initial and end of storage). Data processing and analysis was conducted using SAS 9.1.3 Portable and XLSTAT 2014 (for organoleptic analysis). Results were statistically analysed with Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT).

Organoleptic test

Consumers’ preferences were observed using hedonic test by rating method, where both whole shallot and peeled shallot were assessed. 25 panellists were required to rate their preferences in aroma, colour, general appearance, easiness to peel and general likeness in the scale of 1 (dislike very much) to 5 (like very much). Organoleptic test was done at initial point, one month and 2 months storage. Tabulated data was then analysed using Kruskal-Wallis test in 95% confidence interval, and followed with Dunn’s procedure (Two-tailed test).

Results and Discussion

Colour and texture

Colour and texture are among the important factors influencing consumers’ acceptance to fresh produces. Differences in colour were observed in different samples of shallot (Table 1). However, considering the wide range of L (lightness), a (green-red colour components) and b (blue-yellow colour components), these differences were seemingly still in the same range of colour.

Inconsistencies were spotted in all colour parameters. For instance, 6 hours of 1-MCP treatments showed different L and b values in shallot, but only slightly different in a value.

Whereas 30 hours of 1-MCP treatment caused different only in L value but not in a and b values. Specifically, in b value, a quite wide range of values were observed, showed in four different groups of significance from the DMRT result. This result may be caused by the difference in initial colour of shallot used in the research. The measurement using L, a, and b values was probably too rigorous and organoleptic test was expected to give better confirmation.

Table 1. Colour and texture of 1-MCP-treated shallot

Duration of 1-MCP Exposure (hours)	Colour (Average ± SD)			Hardness Average ± SD (g/cm)
	L	a	b	
0	47.99^{a}±4.39	35.95^{ab}±4.17	11.18^{b}±3.55	1354.85a±367.49
6	43.88^{b}±7.10	34.27^{b}±4.07	10.03^{c}±3.47	1365.69a±347.73
18	48.04^{a}±8.19	34.31^{b} ±5.44	12.12^{a}±2.56	1266.48ab ±293.80
30	43.63^{b}±5.44	38.11^{a}±3.96	13.03^{a}±3.79	1342.65a±342.54
42	48.13^{a}±4.82	35.92^{ab}±5.29	9.01^{d}±4.26	1137.52^{b}±170.49

Note: numbers followed by different letter(s) in the same column show significant difference at α = 0.05. L = lightness (0 for darkest black, 100 for brightest white); a = green-red colour components (negative values indicate green, positive values indicate red); b = blue-yellow colour components (negative values indicate blue, positive values indicate yellow)

The positive a value stood for reddish colour, which is the main unique appearance of shallot.

The colour red in shallot indicates the anthocyanin content, which stability is influenced by light, pH, temperature, sulphite, ascorbic acid, enzymes, etc [11]. Considering that same storage condition was applied to all samples, it was concluded that the differences of a value between different treatments were caused by chemical and enzymes activity in the shallot. The effects of 1-MCP to specific chemical composition and enzymes activity remain to be studied further.

During storage, onion bulbs experiences biochemical changes that cause softening of tissues.

Pectin portion of bulb cell walls was hydrolysed through pectolytic enzymes [12]. 1-MCP treatment on apples had been reported to delay softening of the flesh both in normal atmosphere and in controlled atmosphere [13, 14, 15]. This occurrence was interpreted as the effect of 1-MCP action in inhibiting ethylene production [16].

In the current study, significance difference in hardness was shown by samples that had 42 hours of 1-MCP exposure. Higher hardness value in this measurement showed harder texture of shallot. According to Purwanto *et al.*, [17], the decrease of shallot quality can be indicated by increased water content (watery flesh due to microbiology or mechanical damage) or by decreased water content (empty or hollow). It was observed in this study that the 42 hours-treated shallot had softer texture due to the maintained moisture content.

Proximate composition

Changes in water and carbohydrate contents are among the effects of biochemical changes in onion bulbs [18, 19]. Moisture contents of treated shallot observed in the current study changed in the similar manner, and significantly different changes on non-treated shallot, especially after 48 days storage (Fig. 1). The non-treated shallot showed more fluctuating changes compared to that of the 1-MCP treated ones. This indicated that respiration rate was more actively occur in shallot without 1-MCP treatment. This observation was relevant with the observation on respiration rate (CO_2 production) and ethylene production by Kailaku *et al.*, [8] where 1-MCP treatment succeeded in inhibiting the respiration rate and ethylene production of shallot.

According to SNI 01-3159-1992 [20], accepted water content for shallot is 80-85%. As presented in Fig. 1, most of the 1-MCP-treated shallot had water content in this range, whereas non-treated shallot's water content decreased to around 75% in the end of the observation.

Moreover, shallot that was exposed with 30 and 42 hours 1-MCP showed the most stable moisture content throughout storage, with moisture content between 80-85%.

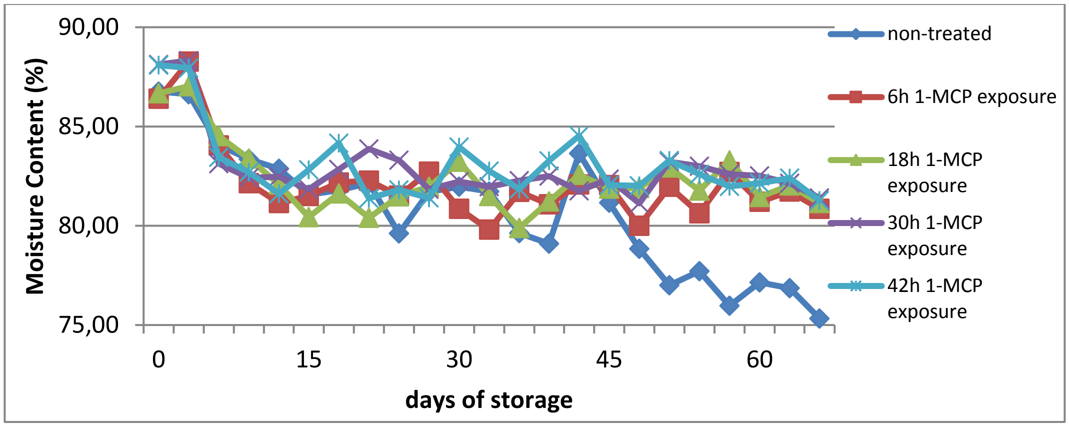

Fig. 1. Moisture content changes of shallot during storage

Analysis on other parameters of proximate composition showed increasing composition of ash, protein and carbohydrate, while fat content decreased (Table 2). Ash content indicates the content of mineral in food. Main minerals present in shallot are potassium, iron and phosphor. Fat content observed in this study was considered high compared to that of the study by Sukasih *et al.*, [11], which used the same cultivar with this study (Cv. Bima), with fat content only $0.07 \pm 0.02\%$. This result was closer to the USDA National Nutrient Database for Standard Reference (USDA 2007), i.e., 0.1%. Protein in shallot acts as anthocyanin precursor, so that the content is positively related to the concentration of anthocyanin [11].

Onion bulbs contain water soluble carbohydrates up to 60-80% of the dry weight, i.e., fructose, glucose and sucrose. The tendency of carbohydrate content to increase or decrease during storage had been reported differently in several studies, and it was suggested that differences can be influenced by the ratio of glucose, fructose, and sucrose concentrations which differ between cultivars [21].

Table 2. Ash, protein, fat and carbohydrate content (%) of 1-MCP-treated shallot

Duration of 1-MCP Exposure (hours)	Ash Content Average ± SD (%)		Protein Content Average ± SD (%)		Fat Content Average ± SD (%)		Carbohydrate Content Average ± SD (%)	
	initial	end of storage	initial	end of storage	initial	end of storage	initial	end of storage
0	0.54±0.02	1.01±0.01	2.32±0.32	2.35±0.13	0.43±0.08	0.36±0.09	14.29±0.61	20.95±2.58
6	0.35±0.13	0.69±0.05	1.99±0.16	2.28±0.20	0.60±0.13	0.48±0.14	15.97±0.26	15.69±1.05
18	0.39±0.03	0.68±0.02	2.15±0.14	2.30±0.45	0.38±0.09	0.27±0.13	14.19±1.90	15.59±0.56
30	0.49±0.02	0.74±0.02	2.04±0.15	2.30±0.15	0.38±0.05	0.28±0.12	13.88±0.60	15.28±0.43
42	0.49±0.15	0.88±0.06	2.09±0.25	2.42±0.17	0.41±0.02	0.30±0.11	13.79±1.75	15.13±0.44

Note: numbers followed by different letter(s) in the same column show significant difference at α=0.05.

Organoleptic quality

Due to its importance as flavour in cooking ingredients, one of the determining qualities of shallot is its flavour and aroma. The differences in flavour of onion have been linked to metabolic dissimilarities in the S-alk(en)yl cysteine sulfoxides [22]. Interestingly, storage tends to increase pyruvate concentration, which generates products sharper in aroma and taste compared to the fresh ones [7]. In this study though, organoleptic test was conducted to confirm that 1-MCP treatment did not interfere with the formation of organoleptic characteristics that are important for consumers.

Panellists in the current study gave similar responses for aroma and general appearance of all whole shallot samples (Table 3). Panellists gave "dislike" in aroma to all samples, showing that aroma had started to change in all samples after 2 months of storage. However, all samples were given "neither like nor dislike" for general likeness, and the 42-hours-1-MCP-treated shallot was given 3.55 which is closer to "like".

Table 3. Hedonic quality scores (n=25) of whole shallot whole shallot after 2 months storage

Duration of 1-MCP Exposure (hours)	Aroma	Colour	General appearance	General likeness
0	2.33^{a}	2.88^{a}	3.45^{a}	3.36^{a}
6	2.48^{a}	3.04^{ab}	3.59^{a}	3.43^{a}
18	2.32^{a}	3.05^{ab}	3.43^{a}	3.36^{a}
30	2.40^{a}	3.11^{ab}	3.43^{a}	3.28^{a}
42	2.40^{a}	3.20^{b}	3.55^{a}	3.55^{b}

Note: numbers followed by different letter(s) in the same column show significant difference at α = 0.05; score 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = like very much.

Significant differences were only shown by 42-hours-1-MCP-treated whole shallot for its colour and the general likeness, where the panellists gave higher scores. The difference in colour score of the 42-hours-treated shallot was only different compared to the non-treated shallot, and not with the other 1-MCP treatment durations.

Panellists were also required to peel the shallot and proceed with the same hedonic test for the peeled shallot (Table 4). In this part of organoleptic test, only general appearance was scored similar in all treatments by the panellists. For aroma, panellists gave "neither like or dislike" for all samples, but the longest 1-MCP treatment duration was given significantly different higher score. This treatment was also given significantly highest scores for easiness to peel and general likeness. This data showed a promising result in the utilization of 1-MCP exposure for shallot fresh handling.

Table 4. Hedonic quality scores (n=25) of peeled shallot after 2 months storage.

Duration of 1-MCP Exposure (hours)	Aroma	Colour	General appearance	Easiness to peel	General likeness
0	3.21^{a}	3.35^{ab}	3.59^{a}	3.28^{a}	3.43^{a}
6	3.29^{ab}	3.65^{b}	3.64^{a}	3.21^{a}	3.59^{ab}
18	3.15^{a}	3.41^{ab}	3.64^{a}	3.21^{a}	3.48^{a}
30	3.16^{a}	3.31^{ab}	3.61^{a}	3.17^{a}	3.41^{a}
42	3.63^{b}	3.47^{ab}	3.71^{a}	3.52^{b}	3.64^{b}

Note: numbers followed by different letter(s) in the same column show significant difference at α = 0.05; score 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = like very much.

Conclusion

Generally, it was concluded that 1-MCP exposure positively influenced the physicochemical and organoleptic properties of shallot. Based on the analysis results, 42 hours of 1-MCP treatment was given the best scores from organoleptic test panellists, which was supported by the physicochemical characteristics.

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Pesticide Mixtures Used by Farmers in Shallot-Hot Pepper Intercropping Cultivation System in Brebes, Central Java, Indonesia

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Abstract

Pesticide-use data were collected by using daily farm-records of 15 farmers who carried out shallot-hot pepper intercropping in September 2015-June 2016. Results showed that the total number of sprayings (TNoS) for shallot ranged between 15-21 times, averaging 18 times, while hot pepper was sprayed 23-34 times, averaging 30 times. All farmers used pesticide mixtures for controlling pests/diseases. In shallot, for instance, one farmer in every spray applied fungicide group M3 (dithiocarbonate), two insecticide-products group 3A (pyrethroids), insecticide group 13 (pyrroles) and insecticide group 6 (avermectins). In 60% of TNoS, this farmer added fungicide group M5 (chlorothalonil), and in 70% of TNoS added insecticide group 2B (fipronil). This farmer was not only using two broad spectrum insecticides from the same MoA (3A), but also adding two more specific insecticides (group 13 and 6). In hot pepper, in all sprayings, this farmer applied two fungicide-products from group M3 (dithio-carbamate) and mixed with insecticide products from group 1B (organo-phosphates), 3A (pyrethroids), 4A (neocotinoids), 5 (spinosyns) and 22A (metaflumizone). Furthermore, in 83% of TNoS, farmer added avermectin (6) and chlorfenapyr (13). Findings of this study suggest improper and excessive use of pesticides that might pose hazards to the farm economics, pest resistance, human health and environment. More comprehensive studies and wider farmers' capacity building on alternative sustainable control techniques are urgently needed. In conjunction with those efforts, the government should also regularly evaluate the enforcement and effectiveness of existing pesticide regulations.

Keywords: pesticide; mixtures; intercropping; shallot; hot pepper

Introduction

Shallot and hot pepper are typical low elevation crops in Indonesia, with the two largest harvested areas and the two highest production values of all vegetables. Brebes, Central Java is the first largest shallot and the third largest hot pepper producing area in Java, Indonesia [1]. The cultivation of those two crops in Brebes is quite interesting to notice since at least once a year; farmers grow shallot and hot pepper using intercropping cultivation system [2]. Both shallot and hot pepper are also perceived as high-risk crops. Previous study suggests that yield risk is mainly caused by pests and diseases [3]. Attacks of pests and diseases can reduce crop yield and quality that finally compromise financial returns of farmers; regional and national markets; and exportation/importation [4].

Synthetic pesticides have become an inevitable input and constitute an integral part of crop management practices in Brebes [5]. The lack of environmentally friendly approaches has also left farmers with no option other than using chemical pesticides [6]. Actually, pesticides have demonstrated their value by increasing agricultural productivity, reducing insect-borne and endemic diseases, and protecting crop harvest [7]. However, the increased and indiscriminate use of pesticides has resulted in problems such as pest resistance, resurgence of pests, toxic residues in food, and exposure to human health, water, air and soil, elimination of natural enemies and disruption of ecosystem [8, 9].

Excessive and misuse of pesticides may occur when farmers attempt to control the pest outbreak by increasing the frequency and dosage of pesticides [10]. Most farmers also tend to ignore the recommended pre-harvest interval written on the label in order to keep the good appearance of vegetable products [11]. While in tropical country, farmers usually keep changing their spraying frequency depending on the weather [12]. The other practice that may lead to severe over-dosage and risks of phytotoxicity is the use of pesticide cocktails [13]. The reasons for application of pesticide cocktails are to save time and the common belief that pesticide mixture can give a greater effect in controlling pest and diseases [14, 15]. The purpose of this study was to assess the extent of pesticide mixtures used by farmers in shallot-hot pepper intercropping cultivation system in Brebes, Central Java, Indonesia.

Materials and Methods

Fifteen farmers conducted shallot-hot pepper intercropping cultivation during the period of September 2015 to June 2016 were purposively selected. Each participated farmer received farm-record sheets at the beginning of cropping period. The following information was collected: chemical brand name, applied dosage, applied amount, spraying dates, spraying volume, purchase price of each pesticide, and the number of applications. Based on the recorded spraying dates, the total amount of pesticides per application, spraying interval and the total number of spraying can be calculated. Meanwhile, form the brand name of pesticides, the active ingredient and Mode of Action group according to Insecticide Resistance Action Committee (IRAC) and Fungicide Resistance Action Committee (FRAC) were identified. The hazardous of active ingredients were categorized according the World Health Organization (WHO) classification (Ia=extremely; Ib=highly; II=moderately; III=slightly; U=unlikely to present acute hazard in normal use; and n.l=not listed).

Results and Discussion

Number of spraying, interval and spraying volume

The total number of spraying for shallot is averaging 18 times, while for hot pepper is averaging 30 times. Spraying interval for shallot is averaging 3.1 days and for hot pepper is 3.3 days. Number of fungicides used per application for shallot is averaging 2.5 products, while for hot pepper is 2.3 products. Number of insecticides used for shallot ranges between 2-8 products, while for hot pepper is 5-11 products. The average spraying volume is 650 l/ha for shallot and 701 l/ha for hot pepper.

Pesticide-products, active ingredients (AI), and mode of action (MoA) groups

The average amount of fungicide AI used in shallot is 9.8 kg/ha, while in hot pepper is 12.6 kg/ha. For insecticide, the average amount of AI used in shallot is 4.1 kg/ha and in hot pepper is 10.8 kg/ha. In average, the number of fungicide-products used in shallot and hot pepper is 5 and 4, respectively. Meanwhile, the number of insecticide-products used in shallot and hot pepper is 8 and 9, respectively. In average, farmers used 3 fungicide active ingredients (from 2 different MoA groups) in shallot, and 4 fungicide active ingredients (from 2 different MoA groups) in hot pepper.

In the meantime, farmers used 6 insecticide active ingredients (from 5 different MoA groups) in shallot, and 10 insecticide active ingredients (from 7 different MoA groups) in hot pepper.

Pesticide used and toxicity risks

Both in shallot and hot pepper, most farmers apply class U fungicides imposing very low health risk. In hot pepper, farmers apply higher percentage of moderately hazardous fungicides than in shallot. More than 80% of highly, moderately and slightly hazardous insecticides (Ib, II and III class) are used in shallot and hot pepper. This implies that the use of insecticides may impose higher health risk than the use of fungicides. As compared to shallot, more percentage of insecticides classified as 1B and II are applied in hot pepper. This suggests that in hot pepper, the risk of farmers to be exposed to more toxic insecticides is much higher.

The use of pesticide mixtures

In both shallot and hot pepper, the most frequent fungicide used is from ditiocarbamate group (M3). In the meantime, mostly used insecticide in the two crops is quite different. For shallot, most frequent insecticide used by farmers is from pyrethroid and pyrethrin group (3A), while for hot pepper is from organophosphate group (1B). As shown in Table 1and 2, pesticides used by farmers (Farmer 1 to Farmer 15) for making mixture vary greatly.

For shallot, Farmer 1 (F1), in 92% of total number of sprayings (TNoS) applies ditiocarbamate (M3) fungicide and in 52% of TNoS adds dicarboximides (E3) fungicide. Furthermore, in 92-95% of TNoS, farmer 1 applies a mixture of pyrethroid and pyrethrin (3A), chlorfenapyr (13) and avermectin and milbemycin (6) insecticides, and on several occasions adds spinosyn (5) and diafenthiuron (12A) insecticides. In hot pepper, F1 applies ditiocarbamate fungicide (M3) in all sprayings (100%). Between 93-100% of TNoS, F1 mixes organophosphate (1B), avermectin and milbemycin (6), and neonikotinoid (4A). In 20-60% of TNoS, F1 also adds amitraz (19), spinosyn (5), pyrethroid & pyrethrin (3A) and diamide (28).

Another case is shown by Farmer 2 (F2). In all sprayings for shallot, F2 applies the mixture of M3 fungicide, and 3A, 5, and neonikotinoid (4A) insecticides. There is no alternating use of pesticides. In hot pepper, F2 applies M3 fungicide in all sprayings, and in 53% of TNoS adds benzo-thiadiazole (P1). In 93-100% of TNoS, F2 mixes them with insecticides from 1B, 6, 3A and 4A groups. In 34-41% of TNoS, F2 also adds (19) and (28).

These two examples suggest that there is no pattern in pesticide mixing carried out by farmers.

Variations among them are so high that may imply indiscriminate and careless use of pesticides.

Table 1. Use of different MoA in shallot sprayings (% of TNoS)

Type	MoA	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	mean
F	M3	92	100	95	90	100	100	88	91	100	82	133	86	86	100	92	96
	M5							13		60							37
	E3	52		94			54		90				7		50	92	63
	G1				85	90						39		21		85	64
	C3			24		10					27	28		71			32
	H5								22				43				33
I	3A	92	100	100	88	20	92			190	73	106	72	36	100	116	91
	13	92		60				63		100	64		50	14		38	60
	6	95		95	90	10		13		100	55	17			92	31	60
	2B								64	70	36	28		7	54	38	42
	5	17	100	42	74	10		13			18	11		14			33
	1B					100		13				33		21		46	43
	4A		100		78				36				21				59
	1A						46							7		23	25
	28			95	65				36			6	93		38		56
	12A	50										11					31

Table 2. Use of different MoA in hot pepper sprayings (% of TNoS)

Type	MoA	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	mean
F	M3	100	100	50	100	69	36	85	100	106	74	77	100	95	68	96	84
	M5			15		8			65		10		60		4	20	26
	E3															44	44
	M1			38				50									44
	P1		53				12										33
	G1						4		15		6						8
	C3													10			10
	H5					4								5			5
I	1B	100	93	135		81	16	98	92	100	13	107	100	24		100	81
	6	93	94	27	41	62	40	75	64	83	23	80	60	29	76	56	60
	3A	20	100	100	82	77	4		95	100		67		38	104	80	72
	4A	93	99	58		35	24	95		100	13	37	60	48	36	56	58
	5	40		73		42	20	92		100	32	30			116	12	56
	19	60	34			62	36		60		3		40			24	40
	2B				94	4	32	55				20		14		64	40
	13			23			32		35	83	26	17		33	8		32
	28	20	41		35						29	17	20	43		4	26
	14			15				45			13	57					33
	12A			15					60						60		45
	15			15	8			20			10	7		29			15
	22A								28	106	13						49

All participated farmers (100%) agree that the use of pesticide mixtures is intended to control more than one pests/disease existed in the field. Most farmers (93.3%) also believe that applying mixtures can give greater effect and more rapid knockdown of pests and diseases than single. A significant number of respondents (86.7%) give an impression that they are indirectly encouraged to use mixtures because of easy access and availability of pesticides. With the passage of time the use of pesticide mixtures becomes habitual, and some farmers (80%) even indicate mixtures-use has become a necessity (14, 15). More than half of respondents (53.3-66.6%) suggest that it is less time-consuming and less labor-intensive to mix two pesticide products or more and spray once.

Reasons mentioned by majority of respondents that mixing pesticides saves time and labor, and that it anticipates higher pest/disease control efficacy are in line with some previous research [14, 15].

However, only 33.4% of respondents agree that mixing pesticides is less costly. The remaining respondents argue that reduction of costs is more likely to come from less labor use only, but not always from the use of mixtures, especially when the combined pesticides are expensive ones.

Discussion

Variations in pesticide mixtures are so high that may imply pesticides are mixed without relation or knowledge about effectiveness or combination possibilities. Farmers may bear several problems that they are unaware of. First, farmers are lack of knowledge about the compatibility (physical or chemical or biological conditions that prevent pesticides from mixing properly in a spray solution) [16]. It can reduce effectiveness or damage plants or cause sprayer clogging.

Second, farmers also have no idea about the most adequate proportion of the components in the mixtures since it is more likely determined based on intuition and ignorance. Third, the spraying volume of the mixtures increases when two or more formulations mixed in the field [17]. The crop will most likely receive an overdose of diluents without having any idea about its consequences on produce quality or any negative interaction with the toxicants when there is no compatibility.

Other potential problems need to be considered may include plant injury (phytotoxicity), pesticide incompatibility [18], and antagonism [19]. The other issue that should also be considered is the use of pesticide mixtures with a broad-spectrum of arthropod pest activity and multiple modes of action that may negatively impact biological control agents or natural enemies more so than separate applications of pesticides [20].

In the meantime, pesticide mixtures may be more effective in suppressing pest population due to either synergistic interaction or potentiation between or among pesticides that are mixed together [21]. Pesticide mixtures may also delay the onset of resistance developing in arthropod pest populations. However, there is minimal evidence to support synergism, potentiation, and that pesticide mixtures may actually mitigate the onset of resistance [22].

The effect of pesticide mixtures is unpredictable because differences in the mode of action do not necessarily ensure a lack of common resistance mechanisms and may only reflect the specificity associated with enzymes responsible for detoxification [23]. Because of the difficulty in generalizing results across systems and the potential negative effects of multiple pesticide use, emphasis on minimizing pesticide use is still highly recommended. Furthermore, since the reliability of the pesticide mixture strategy depends on several assumptions, applying pesticides individually, or rotating those with different modes of action or that act on different target sites may be a more appropriate strategy [24, 25].

Conclusion

The implication of mixing practice is that it may lead to excessive and misuse of pesticides. All participated farmers perceive that the use of pesticide mixtures is intended to control more than one pests/disease existed in the field. Majority of farmers also perceive that mixing pesticides saves time and labor, and anticipates higher pest/disease control efficacy. Meanwhile, easy access and availability of pesticides that according to farmers also encourage them to use pesticide mixtures could be considered as a remainder for the government to review the procedure of pesticide registration and distribution.

Based on the unpredictability and potential negative effects of pesticide mixtures, it is recommended that minimizing pesticide use is still the best option to go. Bringing the issue under control and overcoming the dependency of farmers on chemical pesticides will need not only technological, but also social and policy interventions. More comprehensive research on alternative sustainable techniques of pest/disease control should be equipped with effective promotion program to accelerate their adoption at the farmers' level. Government should prioritize regular evaluation of existing pesticide regulations' enforcement and effectiveness. Measures to promote capacity-building among farmers are also required, enabling them to clearly understand pesticide-related regulations, implement sustainable technologies, reduce pesticide use and promote the development of green products for the market.

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WELCOME REMARK
DIRECTOR OF ICHORD

International Symposia On Horticulture: Emerging Challenges And Opportunities In Horticulture Supporting Sustainable Development Goal

November 27-30, 2018, Bali, Indonesia

- **Honorable Secretary General of IAARD**
- **Honorable Colleagues in Ministry of Agriculture**
- **Honorable invited speakers**
- **Distinguished Guests and Participants**
- **Ladies and Gentlemen**

Good Afternoon,
Assalamu'alaikum Warahmatullahi Wabarakatuh,
First of all, let us say Grace to Allah SWT the Almighty God, for all His blessings so we could gather here in International Symposia on Horticulture. It is my pleasure to extent my warmest welcome to all of you to this important conference at the exotic island of Indonesia, Bali.

This conference is organized by Indonesian Center for Horticulture Research and Development (ICHORD), Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, the Republic of Indonesia.

Distinguished Guests and Participants,
The International Symposia on Horticulture is held starting today on 27th of November to 30th of November, 2018 with the following theme: "**Emerging Challenges and Opportunities in Horticulture Supporting Sustainable Development Goal**".

The four-days conference is aimed at capturing advance information and technology innovation to support horticulture sector development. We hope that this conference will provide discussion forum for national and international research scholars, policy makers, scientists, practitioners, extension agents, students and other professionals to address the challenges of horticulture innovation.

Five important issues will be discussed throughout the conference which include; (1) Promote the Exotic of Tropical and Sub Tropical Fruits to Enhance Competitiveness and Penetration into

Modern Markets, (2) Modern and Urban Farming System for Healthy and Safety Vegetable Products; (3) Coloring the World with Ornamental Plant Innovations or Prosperity; (4) Implementation of Integrated Crop Managements to Support Food Sovereignty Based on Bio-industry; and, (5) Emerging Trends of Horticulture Impacting Global Markets: The Socio Economic Studies.

Distinguished Guests and Participants,

This conference is attended by 37 international participants from 14 countries: France, United Kingdom, Ireland, Australia, Nigeria, Pakistan, Japan, Thailand, Malaysia, Philippines, Myanmar, Singapore, Vietnam, Cambodia, and 168 colleagues from all over Indonesia.

On the next 3 days, 10 invited papers and 205 supported papers will be presented, consisting of 132 oral and 71 poster presentations. Participants will also have the opportunity to visit one of IAARD project on Bioindustry in Bali during one day field trip on the third day.

Distinguished Guests and Participants,

Let me take this opportunity to express our high appreciation to our partners: ACIAR, PERHORTI, PERAGI for supporting this conference. I also would like to sincerely thank our honorable keynote and invited speakers and all participants for your active participation and support.

Last but not least, I would like to express my gratitude to the organizing committee whose endless dedication has been priceless to organize this event. I wish you all have a most successful conference and enjoy your stay in Bali.

Finally, I would like to kindly ask Sekretary General of IAARD to officialy open this conference.

Thank you.
Wassalamu'alaikum warohmatullahi wa barokatuh

Director of ICHORD
Dr. Hardiyanto

OPENING REMARKS
BY
SECRETARY GENERAL OF IAARD-MOA

International Symposia on Horticulture:
Emerging Challenges And Opportunities In Horticulture Supporting Sustainable Development Goal

November 27-30, 2018, Bali, Indonesia

- **Honorable invited speakers**
- **Honorable Colleagues in Ministry of Agriculture**
- **Distinguished Guests and Participants**
- **Ladies and Gentlemen**

Good Afternoon,
Assalammu'alaikum wa Ramatullahi wa Barakatuh,

I would like to begin by giving thanks to God Almighty Allah Subhanahu Wa Ta'ala; His blessings are evident as we gather here today to take part in **"International Symposia on Horticulture: Emerging Challenges and Opportunities in Horticulture Supporting Sustainable Development Goals"** in Kuta, Bali, Indonesia.

Distinguished Participants, Ladies and Gentlemen

According to United Nation projections, the world's population could reach 9.15 billion by 2050, creating a 60 percent increase in demand for food. Hence, the demand on sustainable and easy access to safe, fresh, nutritious, tasty, and convenient food to maintain a healthy and active lifestyle will also be increased.

In order to full fill the demand, Indonesian government, through Ministry of Agriculture, has set strategic targets to achieve food self-sufficiency by: (1) increasing products value added, competitiveness, exports, and import substitution; (2) provision and improvement of bio-industrial raw materials and bioenergy; and (3) improvement of farmers welfare.

Distinguished Guests, Ladies and Gentlemen

Horticulture sector with its high economic value plays an important role to achieve the above goal. However, this sector still faces many challenges. Some of the issues we need to address are how to increase competitive advantages in horticulture products, and how to create an efficient and effective chain management from land to the airport or marketing centers. Another challenge is creating and maintaining strategic linkages in local, regional and international network.

In case of horticulture export development, there is growing concern about the safety of horticultural products. Exported products are often rejected by the importing countries due to the presence of unwanted contaminants. Development of rapid, simple, and relatively accurate detection methods for contaminants as well as their prevention and elimination technologies are necessary.

Distinguished Guests, Ladies and Gentlemen

I applaud the collaboration of you all to convene this four-days event. From our discussion, I expect that we can share knowledge and information to strengthen horticulture development. I hope the results of this conference would become valuable references to address the emerging challenges and opportunities in horticulture to support sustainable development goals.

In this regard, I would like to invite all stakeholders to actively participate and support this important conference. With *Bismillahirohmanirohim*, it is now my distinct pleasure to declare this symposium **open**. I wish you a fruitful and successful conference.

Thank you
Wassalamu'alaikum wa rohmatullahi wa barokatuh

Director of IAARD

IV. ISH AGENDA

Time	Session	Topic
	Day 1 (27 November 2018)	
10.00-12.00	Registration	
12.00-13.00	Lunch at Kunyit Restaurant	
	Opening Ceremony	
13.00-14.30	Opening ceremony 1. Traditional dance (5’)	
	2. MC (5’)	
	3. Report of Organizing Committee by Director of ICHORD (10’)	
	4. Welcome Speech by Governor of Bali (10’)	
	5. Opening remark by DG of IAARD (10’)	
	6. Dr. Irene Kernot (Research Program Manager Horticulture of ACIAR) (20’)	Improving the Productivity, Profitability and Sustainability of Fruit Vegetable and Ornamental Crop Production in Development Countries
14.30-15.00	Photo session, Poster Session and Coffee break	
	Moderator: Dr. Catur Hermanto, Notulen: Hafith Furqoni, MSi	
15.00-15.20	Pepijn Schrenemachers	Vegetables for Better Nutrition: The Need to Target Demand as well as supply
15.20-15.40	Randy Stringer	How do Smallholder Farmers of Horticulture Product in Asia Access the Modern Markets
15.40-16.00	Bahman Amiri Larijani	Protected Farming on Horticulture
16.00-16.20	Tomohiro Kamogawa	History and Challenge in Joint Plant Breeding to Bring Impact to Global Market
16.20-16.40	DG for Horticulture (20’)	National Policy for Horticulturee Supporting Sustainable for Development Goals
16.40-17.15	Poster session	

DAY 2 28 November 2018					
	Promote the Exotics of Tropical and Sub Tropical Fruits to Enhance Competitiveness and Penetration into Modern Markets (50 papers, 34 orals, 16 poster)	Modern and Urban Farming System for Healthy and Safety Vegetable Products (76 papers; 48 orals, 28 posters)	Colouring the World with Ornamental Plant Innovations for Prosperity (16 papers; 10 orals, 6 posters)	Emerging Trends of Horticulture Impacting Global Markets: The Socio-Economic Studies (53 papers, 36 orals, 17 posters)	ROOM FOR POSTER SESSIO N
08.30-09.30	1. Head of ITFRI (20') 2. Stefano De Faveri 3. Prof. Darda Efendi 4. Dr. Guinevere Ortiz	1. Head of IVEGRI (20') 2. Prof. Michael Boehme 3. Steven de Neve	1. Head of IOCRI (20') 2. Margareth Serekh 3. Head of IATT	1. Allain Rival 2. I Made Suparta	
09.30-10.00	Discussion				
10.00-10.30	Poster Session and Coffee break				
10.30-11.30	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	5 paper (7') +(25') discusi	
11.30-12.30	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	5 paper (7') +(25') discusi	
12.30-14.00	Lunch at Kunyit Restaurant				
14.00-15.00	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	Implementation of Integrated Crop Managements to Support Food Sovereignty Based on Bio-industry	5 paper (7') +(25') discusi	
15.00-16.00	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	1. Head of 2. Prof. Suzuki Katsumi 3. Dr. Nurul Humaida (PERAGI) 4. Olovier Gibert	5 paper (7') +(25') discusi	
16.00-17.00	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion	5 paper (7') +(25') discusi	5 paper (7') +(25') discusi	
17.00-18.00		5 paper (7') + (25') discusion	Closing		

Day 3 29 November 2018 (FIELD TRIP)					
07.00-09.30	Start from Hotel to Bio Industry, Atapan				
09.30-10.30	Bioindustry				
10.30-11.00	Bio industry to Ulun Danu Baratan				
11.30-13.00	Lunch at Daya Tarik Wisata, Ulun Danu Beratan, Bali				
13.00-15.30	From Baratan to Krisna shoping center				
15.30-16.30	Krisna				
16.30-17.00	Back to Hotel				
	Day 4, 30 November 2018				
07.30-08.30	5 paper (7') + (25') discusion	5 paper (7') + (25') discusion		5 paper (7') + (25') discusion	
08.30-09.30	4 paper (7') + (25') discusion	5 paper (7') + (25') discusion		6 paper (7') + (25') discusion	
09.30-10.30	Closing	5 paper (7') + (25') discusion		Closing	
11.00-11.05		Closing			
11.05-13.30	Friday Prayer and Lunch				

MINUTES OF MEETING

Endang to Stefano
Solution for nutrient efficiency?
Session 2:

1. Presenter Gina
   Question: Stefano
   Nitrogen uptake high, productivity low in Pacet because the slope, grow in rainy season so erosion, management of nitrogen application. Apply 3-4 time in early crop so erosion
   Phospor, 8 pp very organic type

   Question: Yusdar
   pH low in Pacet but no lime application, vice versa in Cirebon? How to solve?
   Solution:
   Farmer knowledge should be increased, extension
   Economic analysis should be done as evidence
   Alluvial, apply dolomite and Kaptan in Cirebon

2. Presenter: Wiwin
3. Presenter: Desak
   Q: Try in the lab or in the real condition, is that effective in the field
   A: This is still a basic research and will be developed

4. Presenter: Arif
   Q: pest outbreak caused by particular number of bio-control agent, do you know to examine it
   A: interesting study on how many populations to maintain equilibrium, next step will try to see it and other thing involve with it

5. Heni P
   Q: Ali Asgar to Heni
   No chemical analysis,
   Answer: this research is only in survey, no chemical analysis. Will do more research

Sesi 2:

1. Sari Girsang 1
   Q
   A
2. Sari Girsang 2
   Q: Stefano: Shallot yield low
   A: dry bulb 10 tons/ha, climate effect

3. Kristamtini1
4. Kristamini1
5. Wicaksono
   Q: Using cabrio, add bacteria? Similar with 24D
   A: Cabrio have auction

Afternoon session:

1. Ali:
   Q:
   A:
2. Musfriah:
   Q:
   A:
3. Setyorini
   Q: Based on the skin color test, which cultivar preferred by farmer. Batu ijo is still the best one in the high land
   Did you use color chart?
   A: Skin color is this one characteristic to be checked. Batu ijo is till the great one. Panjatan is the better.
   Use colorimeter but not comparing to color chart
4. Rahmansyah
   Q:
   A:
5. Asih
   Q: What is the recommendation for farmers?
   A: The final goal is to create VUB resistant against fruit fly. Next year new variety will be released
6. Selangga:
   Q: Silica?
   A: Granule silica, gravimetric method for total silica
7. Rinda Kirana
   Q: What volatile chemical?
   A: volatility compound correlated with susceptibility
   It written in another article, terpene
8. Redy
   Q:
   A:
9. Fadila
   Q:
   A:
10. Miranti:
    Q from Asih, seed from Ivegri, from which generation? Do not call G1 just from G0
    Cingkariang, 120 mature the other 100 mature
    Research in potato, granola should put in research
    2. Only temperature observed, not add correlation
    A: Yes, used seed from IVEGRI, G0 generation. Not directed planted from plane
    Granola was not used because low productivity
    2. Soil humidity, yes…no correlation tested

Afternoon:

1. Lia
   Q:
   A:

2. Retno Pangestuti

Q:

A:

3. Endang:

Q: Setjajit: comparing shallot

A:

4. Ema Lindawati:

Q:

A:

5. Sarci:

Q Finding is good, what the effect of treatment.

A: finding to find combination, plan to use micoriza

Incorporate michoriza will be interesting in the future

6. Saraswati

Q

A

7. Witono Adiyoga

Q: Suryawan: have experience, in Brebes 2 years 3-7 kinds pesticide used in shallot. Now mixture high. No change attitudes. How come? More than 20 years

Better used rotation pesticide

A: Same question.

Very difficult to change farmers attitude

8. Dermiyati

Q:

Stephen Harper;

Long term experiment will be beneficial

What nutrient content

Experiment design, why chose that nutrition

Suryawan: combination of organic and non-organic 50:50, why?

A:

1. Research was done in 2012, developing in 2014.
2. Reformulation, > 1 %, P > 5 %, 1,2 % for K, just like NPK
3. 70/2011, related to organic matter for checking affectivity. Should follow recommendation
4. Di Lampung, Ultisol not fertile, need to be improved.
5. High concentration

9. Nurul Aini

Q: Nurul: good Result, is there any long-term road map?

Suryawan: What kind of recommendation, combination PGPR + micorhiza?

A: We have management nutrition roadmap.

Hydroponic is about quality

EC in vegetative

10. Setyajid

Q: Sodium bisulfate, it is safe for treatment

A: For now, it is still allowed

Be replaced with citric acid

FINAL MINUTES OF MEETING

A. Irene Kernot

Title: Improving the productivity, profitability and sustainability of fruit, vegetable and ornamental crop production in developing countries.

I. **Why** we need good research for development

- Perspective of the end user of the research → How can we grow more and healthier food, distribute and share it better, waste less and do all that in more difficult climates using less land, water, energy and nutrients.

- This question reflects the link between agriculture and health; that most of us now live in cities and that production is not equally distributed around the planet; that resources are limited and that climate change is real.

- As researchers in horticulture are part of the global food system

- The number of people suffering from acute hunger is rising again after starting to fall. Over 800 million do not have enough food to eat. As many as two billion people suffer from micronutrient deficiencies, with the lifelong impacts that result. And added to this more than two billion people are overweight and with that the incidence of non- communicable diseases such as diabetes and heart disease is growing.

- The food system is broken and needs to be fixed.

- Research by the University of Guelph linking global production with global nutritional needs showed that we already produce more grains than we need for a healthy diet, three times as much oil and fat, a lot more sugar, but only two thirds as much protein and one third of the fruit and vegetables we should be eating.

- Work is needed to match demand to health and wellbeing and develop closer linkages between Departments of Agriculture and Departments of Health

II. The ACIAR model for **how to** create an effective research program

- Hand-in-hand with the challenge is the opportunity. Horticulture is central to the opportunity. The world needs to grow and eat more fruits and particularly more vegetables.

- ACIAR translate questions into actions. Industries and farmers can implement and was shown in ACIAR mission statement

- ACIAR was established in 1982. ACIAR is an investor in scientific research for agriculture. The organisation sits not with the Department of Agriculture but within the foreign affairs portfolio and answers directly to the Foreign Minister.

- In this role, ACIAR broker research between Australian research organisations and relevant organisations in ACIAR partner countries to build knowledge to support the objectives of Australia and the partner countries in which we work. The work is aimed to contribute towards these UN sustainability goals.

III. **What** two of our research projects are doing to provide science to support changes to production system paradigms.

- UN Sustainability Goals.
  - no poverty
  - zero hunger,
  - good health and wellbeing,
  - gender equality,
  - clean water and sanitation,
  - decent workload and economic growth,
  - industry innovation and infrastructure,
  - responsible consumption and production,
  - climate action,
  - life below water,
  - life on land,
  - partnerships for the goals.

- ACIAR Strategic Objectives

1. Food Security and poverty reduction – where our productivity and profitability related projects sit
2. Natural Resources and Climate change – such as working on improved irrigation management
3. Human Health and Nutrition – School feeding projects and increasing the use of indigenous plants and diversity of crop systems.
4. Gender Equity and women's empowerment – understanding women's role and creating projects that include women and also young people such as the family farms extension program in PNG
5. Inclusive value chains – recognising the whole chain such as the mango processing project workshop over the weekend
6. Capacity building – integral to everything we do

- In ACIAR this strategic is achieved through 10 core research programs supported by 6 cross cutting research themes. The cross cutting themes reflect that our research is applied within complex biological, economic and social systems often best addressed by multidisciplinary projects. Such as the new fish rice system research underway

1. Agribusiness
2. Crops
3. Horticulture
4. Livestock
5. Fisheries
6. Forestry
7. Water and Climate
8. Soil and Land Management

9. Social Sciences
10. Impact Evaluation

- With cross cutting themes

1. Farming Systems Analysis
2. Gender
3. Climate Change
4. Economics and Policy
5. Nutritionally Sensitive Agriculture
6. One Health

- ACIAR operate in 36 countries and through 10 country offices supporting over 200 projects with over 100 partner research agencies. Collaboration is critical, both to find enough researchers to do the work but to spread the capacity for research across the region and to do research that is locally relevant.

- Each region has a research strategy developed in participation with that regions' government, industry and research institutions.

- Every point of the research procurement process is collaborative to ensure that ACIAR builds strong partnerships with commitment to the project and its planned outcomes.

- The strategy is now to invest within larger longer term programs of work to provide greater security for researchers and the ability to invest in projects that have bigger targets.

- The partnerships are also maturing as more as countries develop capacity to invest in their own research and we are bringing in new partners from private industry who also increasingly understand the value of research for development.

- ACIAR invests in a capacity building program that includes support for postgraduate training and training in research leadership. We are developing a strong alumni network throughout our regions and we are committed to supporting that network to create lasting personal relationships that will go beyond the projects and create an environment for ongoing collaboration.

- As much as ACIAR need farmers to have hope and confidence about their future, ACIAR horticulture scientists need to see this line of work as interesting and rewarding. ACIAR want many young people to choose horticulture research, and research for development as their preferred career.

- ACIAR also need to let the community know what ACIAR are doing through effective outreach programs that promote the value of investment in research. When government and industry has a choice about how to invest, the better we can show how horticulture links with food and health, and good business, the more attractive we can be to our investment partners.

- Two projects that the Horticulture program in ACIAR is funding, namely: One is a tree crop intensification project and the other is addressing disease management in banana. More information on all of our projects is available on our website.

- ACIAR has funded projects to manage and control the spread of Fusarium disease for over 10 years in Australia, Indonesia and the Philippines, but there is still some way to go. In a system where a monoculture of a single variety of banana has tipped the balance towards the pathogen, we need to change the way we grow bananas. The question that we need to answer is;

- How will I continue to farm in the presence of TR4? Because the disease is spreading and there is no silver bullet solution. Despite being first identified in the early 90s no truly resistant commercial cultivar has been identified. There are varieties that are more resistant but less commercially acceptable and so they are used as a last resort. Thus inoculum builds up in the system to a point when even the partially resistant lines struggle to survive when growers finally turn to them. So we are looking to develop a suppressive system that will no longer favour the pathogen and, through increasing the microbiological diversity in the soil create an environment where disease inoculum is lowered and more partially resistant lines can be grown successfully. These changes may require productivity trade-offs. Research in Australia demonstrates that the high nitrogen fertilisation of banana necessary for high yield will shift the balance in favour of the pathogen in a banana with fusarium system. Reducing nitrogen reduces disease competitiveness but also yield. We need to increase research on soils to create the balance in the soil necessary for disease suppression. The new sciences of proteomics and metabolomics are giving researchers new tools to help growers shift management practice towards a soil microbiome managed for crop health support.

- ACIAR is funding the research to better understand the role of the soil microbiome and how to manipulate it because we cannot afford to ignore this resource as we gain understanding of the profound effect of the soil community on the health of the plant. From a better understanding of the soil I go to a project that is better understanding and manipulating the plant.

- ACIAR mango research goes back 36 years since the beginning of the organisation. In that time 42 projects have tackled a very broad range of issues.

- Recently ACIAR commenced investment in a Small tree High Productivity research project initiated in Queensland Australia. This team wanted to replicate the apple story where apples were able to transform their system from large vase shaped trees yielding 30 t/ha or less to 130t/ha from small trellised trees through the application of improved genetics, vigour reducing rootstocks and intensive tree management.

- Can a tropical tree adapt to intensive tree management? Traditionally mango trees are grown widely spaced and allowed to grow into very large trees with all the associated difficulties in pest management and harvesting. These large trees are also more prone to damage in severe storms. The team tried training mangoes and now only four years later the results are looking promising. The team is taking mango trees that typically yield between 4 and 15 t/ha to nearly 50 t/ha. The trees are smaller and can be easily harvested and managed from the ground. As the trees are trellised they also benefit from improved cyclone or typhoon resilience. These systems also lend themselves to reduced labour and other innovations in crop and pest management.

- I hope that this work will now inform the future of tropical tree fruit research for productivity gains within the ACIAR research portfolio. Deploying improved genetics and crop management we will look to research to achieve more efficient and effective use of a limited land resource, more efficient and effective use of light and water and more efficient and effective use of increasingly limited labour. So to the delegates here who are working on the challenge of improving productivity, profitability and sustainability you also contribute to the global challenges through the projects you do.

- Horticulture must be linked closely to planetary and human health.And every farmer and business in the supply chain is important. System improvements will be delivered not through your actions but by the actions of farmers in farming systems, and their supply chain partners, who grow and distribute the horticulture products we research and they need to be able to afford to implement changes. To meet the challenge we need to build the strong and lasting research partnerships that conferences such as this support. And we need to support the future by engaging the next generation.

B. Ir. Sri Wijayanti Yusuf, M.Agr.Sc.

Title: National Policy on Horticulture for Supporting Sustainable Development Goals

Sustainable development goals:

1. No poverty
2. Zero hunger
3. Good health and well-being
4. Quality education
5. Gender equality
6. Clean water and sanitation
7. Affordable and clean energy
8. Decent work and economic growth
9. Industry, innovation and infrastructure
10. Reduced unequalities
11. Sustainable cities and communities
12. Responsible consumption and production
13. Climate action
14. Life below water
15. Life on land
16. Peace and justice strong institutions
17. Partnerships for the goals

National horticulture policy and priority commodities

1. Chili and shallot to supporting quality seed, pest and disease control, processing, marketing
2. Garlic and potato: increasig competitiveness and added value of horticulture products
3. Fruits and flowers: building horticulture cluster, economies of scale, upstream-downstream, undustrialization

The national policy direction for chili and shallot

- Chili and Shallot influenced the economic inflation
- Planted through out Indonesia, small holder farmers
- Stable supply all the year, increasing demand during Eid Mubarak, Christmas, and new year.
- Planting off season
- Supply through out the year
- Main spice of Indonesian foods, no alternative, can not be replaced by other commodities
- Consume everyday in fresh: Chili: 1,77 kg/capita each year. Shallot: 2,57 kg/capita each year
- Many islands, high distribution cost
- Processing and marketing

10 ways to secure and stabilze chili and shallot supply

1. Balancing supply by extensification the area outside Java
2. Intensify the technology of cultivating in Java as a central production area
3. Increase the capability of farmers outside Java
4. Using true shallot seed (TSS) to decrease production cost up to 65%
5. Integrated Pest Management (IPM)
6. Manage cropping patterns, times and regions
7. Build the horticulture auction market
8. Processing products
9. Storage technology to make the product more durable and last longer
10. Increasing export of shallot

The national policy for garlic

- Early 1990s, Indonesia was self-sufficiency in garlic, only 5% import
- Recent data shows, **95% import** and only 5% from domestic supply
- Begin to plant garlic in suitable area to achieve self-sufficiency in garlic 2021
- **Current problem: Seed production** to fullfill farmers need.

The national policy direction for potato

- Total production (2017): 1.164.738 ton
- Grow on high land areas (2017): 83.172 hectare
- Potato for chip and french fries industry still imported
- Target 2020 self sufficiency

The national policy direction for fruits

- Extention area for citrus: low land and high land
- Low land: tangerine, high land: mandarin.
- Encourage farmers in highland area to grow more mandarin citrus
- Existing area → Improving cultivation management: Fertilizing, prunning, pest and disease management, post harvest handling
- For export purposes: Good Agricultural Practices (GAP)
  - Fertilizing
  - Prunning
  - Pest and disease management
- Good Handling Practices (GHP)
- Farm Registration

- Packing House Registration
- Regular surveillance

C. Pepijn schreinemachers

Title: Vegetables for better nutrition: the need to target demand as well as supply

- Vegetables are mankind's most affordable source of vitamins and minerals needed for good health. WHO recommends at least 200 grams of vegetables (and another 200 grams of fruit) a day.
- Fruit and vegetable intake levels are inadequate globally. Low intake of fruit and vegetables is among the top 10 risk factors for mortality in the world. Vegetable consumption does not automatically go up with rising incomes – it may even go down.
- Improving nutrition requires both interventions to reduce the intake of harmful foods (high in saturated fat, added sugar, and salt) and increase intake of healthy foods (e.g., fruits and vegetables)
- Drivers of low fruit and vegetable intake:

Supply constraints:

- High prices (affordability)
- Seasonal unavailability
- Nutritional value of some vegetables

Demand constraints:

- Low awareness
- Negative perceptions
- Lack of trust in product quality

Supply-side:

1. Promote nutrient-dense veggies

Micronutrient	Cabbage	Moringa	Amaranth	Aibika	Sweet potato leaf
Beta carotene, mg	0.00	15.28	9.23	5.11	6.82
Vitamin C, mg	22	459	113	82	81
Vitamin E, mg	0.05	25.25	3.44	4.51	4.69
Iron, mg	0.30	10.09	5.54	1.40	1.88
Folates, mg	NA	93	78	177	39
Antioxidant activity, TE	496	2,858	394	560	870

2. Increase supply in off-season

Impact of training vegetable farmers in off-season production in Bangladesh, 2016

Outcome indicator	Impact	Sign.
Total income during off-season (*kharif*)	48%	$p<0.05$
Quantity of pesticides used	56%	$p<0.05$

Supply & demand-side: Home gardens

Demand:
1. School gardens
2. School meals programs

Conclusion
- Current food systems are not resulting in increasingly healthy, high-quality diets.
- Vegetables are nutritional powerhouses, key sources of micronutrients needed for good health. They add diversity, flavor, and nutritional quality to diets.
- But focusing on increased production is not enough.
- Vegetable demand must be nurtured alongside supply.

D. Randy Stringer

Title: How Smallholder Horticulture Farmers in Asia Access Modern Markets: The Indonesian Case

- Small holders access modern markets via specialized wholesalers, processors, export programs and projects
- But, not that many are linked yet, why?

a) Small farmers' weaknesses
b) Scale and shortage of capital
c) Lack of access to technology and knowledge
d) Need to achieve minimum quantities
e) Consistency, traceability and quality standards

Limiting factors in Indonesia's small farm sector:
- Stagnant production and yield gaps
- Rising land and labour costs pressures
- Land trade off: high value crops vs stables
- Trade trade-off: staples vs high value imports

Catching up in 3 ways:

1. Infrastructure
2. Approach from production to food system
3. Transitioning our research: Breeding, extension, policy

Rebalancing the Policy Space for Food Systems

- Dealing **both with supply and consumer demand – with** interventions throughout food value chains
- **Multi-sectoral** – agriculture, health, industry, environment, transport, and trade
- **Spatially differentiated** – taking into account natural resources, demographics, institutional capacities and food preferences
- More **commodity neutral at national level** – staples, secondary foods, high value foods
- Providing **core public goods** and services while facilitating private investment and initiative

E. Bahman Amiri Larijani

Title: Protected Farming on Horticulture Experiences in Iran

- Horticultural crops Cultivation area in Iran
  - ➢ According to the survey conducted in 2015, the crop area of the total agricultural production in Iran is 16,467,000 hectares.
  - ➢ In 2015, the horticulture area of the country was about 2.68 million hectares, about 86% of which was irrigated and the rest was dryland.
  - ➢ The country's horticultural products were estimated at 19.38 million tons, of which 94.19 percent were irrigated and the remainder was rain-fed.

- **Vision of Ministry of Agriculture**

➢ Intensive cultivation by shifting to greenhouse crops
➢ Reduce water usage by 30% by transferring from open field to indoors.
➢ Increase productivity and reduce water consumption by promoting new irrigation systems.
➢ Agricultural Mechanization
➢ Food safety and sustainable agriculture

- Protected Agriculture (PA) is defined as "modification of the natural environment to achieve optimal growth" (Jansen and Malter 1995). The definition of protected agricultural production includes "mulches, row covers, shade structures, greenhouses, etc. Any type of method or structure used to extend the growing season of plants". **Using these definitions**, estimates of the global production area for both "Greenhouse" and "Protected Agriculture" have been made, using government statistical references as available, updated to January 2018.

- **Why Protected Agriculture?**

- Climate change: The amount of carbon dioxide and methane in the air increased by 30% and 150%, respectively, Compared to the pre-industrial revolution.

Greenhouse vs Protected Agriculture

- Since the release of the 2018 World Greenhouse Vegetable Statistics publication, several questions have been asked regarding the types of structures used in “Greenhouse” vs. “Protected Agriculture” statistics.
- The answers are related to the actual definitions used.
- For greenhouse vegetable production, the most commonly used definition is: “only permanent structures covered with glass or plastic, and generally excluding simple high or low tunnels, shade houses, etc.” Other definitions add to the permanent structure requirement “with climate controls”, and “with computerized irrigation systems”.

- Global Greenhouse Vegetable Area: 497,815 hectares (1,230,128 acres). Global Protected Agriculture Vegetable Area: 3,414,353 hectares (8,437,050 acres)
- The term protected cropping refers to the use of glass or polythene to give improved conditions for the crop grown inside. Crops that benefit from protection include: Fruits, Vegetables, Ornamental plants, Medicinal Plants
- Protected agriculture offers many advantages, such as:
    - Large increases in yield, produce quality and income
    - High water productivity, saving significant amounts of water
    - Significant reduction in pesticide use – lower production costs, healthier produce
    - Year-round production, allowing farmers to take advantage of market seasonality and higher prices

- Water reduction strategies using mulch system. Temperature challenge using double cropping

- Protected system:

- ❑ Light (By its intensity, duration, direction and wavelength) can influence plants different responses in photosynthetic rate, growth, leaf morphology, dry weight allocation patterns, phenology, crop production and quality.
- ❑ Very strong sun isolation during summer day can cause significant damages in every crop production especialy if it is followed by excessive light, drought, and low relative humidity.
- ❑ In recent years with climatic changes and extreme weather conditions producers all over the world experience problems, not only which sunburns which affect crop quality, but even with other problems connected to plant development.
- ❑ Today modern agriculture offers net shading protection with more advantages for plants, producers and consumers.
- ❑ Netting in different colors and shading factors not only protects from sunburns but as well affect environmental modification (like humidity, temperature) by creating comfortable microclimatic conditions for plant development.
- ❑ New innovative screens can increase the relative proportion of diffuse (scattered) light as well as absorb various spectral bands, thereby affecting light quality.

- ❑ Managing light quality, we can stimulate plant growth and cropping, better taste and more intense colors of crops and more pleasant work environmental.

- INSECT PESTS
    - Fruit and vegetable crop production is limited by insect pests and insects transmit virus pathogens;
    - Damages caused by insect vectors and plant viruses are economically significant;
    - Control of pests usually implies intensive use of insecticides;
    - Extensive use of insecticides adversely affects environmental and people health;
    - Climatic changes will contribute to worldwide migration or expansion of the ranges of many insect pest species;

- DAMAGES
- Direct damage: pests feed on marketable parts of plants causing primarily quality but as well quantity losses.
- Indirect damage: pests feed on non-marketable parts of plants causing yield reduction.
- Damage caused as vector diseases: insect transmits pathogenic viruses causing both yield and quality losses.
- Damages by contamination: presence of pests or some insect parts decreases market value of product

- INSECT SCREENS
- Effective pest management tool against various insect pests
- As physical barrier it reduces pest access to plants and transmission of viral diseases
- It keeps inside beneficial insects (pollinators and natural enemies)
- It creates better microclimatic conditions comparing to plastic film
- It stands to reduce the use of pesticides leading to healthier environment, quality and safe food.
- Solutions for field and protected crop production
- A greater ventilation in warm climates and effective protection even against smallest insect pests

F. Tomohiro Kamogawa

Title: History and Challenge in Joint Plant Breeding to bring impact to Global Market

- The situation of Ornamental Plant Genetic Resources
    - Convention on Biological Diversity (CBD) and Nagoya protocol
    - CBD enters into force on December 1993
    - Nagoya protocol enters into force on 12 October 2014
    - **Japan ratified** Nagoya Protocol on **May 2017**

- Objectives of CBD:

1. To conserve biological diversity
2. The use biological diversity in a sustainable fashion
3. To share the benefits of biological diversity fairly and equitably

- Importance of Genetic Resources. What is genetic resource, (wild plants, land races, traditional varieties). Breeding material (plant shape, flower color, taste, resistant to disease) adding high value to generate profit

- Joint developmet with INTA, Argentina

Cooperative Collecting Expedition and Evaluation Program for New Ornamental Plants *CEE*. Ornamental breeding programs: the germplasm is the raw material for the genetic improvement

3. Access to Genetic Resources of Indonesia for "SunPatiens". Impact of the "SunPatiens" in the Global Market. Impact of the "SunPatiens" in the Global Market
   a. Improve current established ornamental plants crossing with newly collected native plants. ex. SunPatiens (New guinea Impatiens),

New interspecific petunia

   b. Create new ornamental plants using newly collected

native plants.
ex. Calibrachoa, Mecardonia

Question and Answer

Q: How to produce horticultural product from small farmer and bring to the market. How related a capital to produce a high product?

A: Focus to farmer product so the farmer can access the capital easily. They do not need focus to all comodities to the farmer but more to focus to comodities that farmer develop.

Q: Regarding ornamental plants. How you determine that flower have a high impact to the farmer?

A: One of benefit is with Indonesia government to have a collaboration with small farmer. For example, one of collaboration with Argentina, they have opportunity to develop new variety. New impatients before was weeds and resistant to desease and can grow in low temperature. Native plant has many potential so it can develop to new variety.

Q: How does horticulture contribute to sustainable agriculture?

A: Agricuture can provide income, jobs for the farmers and it can be sustainable, food security is not only providing micro nutrient, sustainable agriculture is produced varies product.

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PROCEEDING

INTERNATIONAL SYMPOSIA ON HORTICULTURE

The International Symposia on Horticulture (ISH) was held for 4 days from 27 until 30 November 2018. This Symposia was organized by Indonesian Center for Horticulture Research and Development (ICHORD) under Indonesian Agency for Agricultural Research and Development (IAARD) Ministry of Agriculture, and was supported by Australian Center for International Agricultural Research (ACIAR), Indonesian Horticulture Association (PERHORTI) and Indonesian Agronomy Association (PERAGI). Theme of ISH was "Emerging Challenges and Opportunities in Horticulture Supporting Sustainable Development Goals". The outputs of the Symposia were (1) elevation a number of new ideas of horticulture innovation development, (2) spread information related to technology of horticulture innovation among horticulture scientists and practicians, and (3) increase collaboration among the various parties to develop agrihorticulture networks.

This proceeding contains 5 main topics such as tropical and sub-tropical fruits, vegetables, ornamental plants, socio economics studies and integrated crop managements, which have been selected and reviewed by editors.

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