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FOREWORD

The International Seminar on Tropical Horticulture 2016 was held in IPB International Convention Center, Bogor, Indonesia 28 – 29 November 2016. This seminar was organized by Center of Excellence for Tropical Horticulture Studies (PKHT), Center of Excellence in University (PUI-PT), Bogor Agricultural University (IPB), and supported by an excellent collaboration with International Tropical Fruits Network (TF Net).

We're very glad to know the fact that the seminar displayed a very wide discussion about tropical horticulture with delegates from 5 countries (Taiwan, Thailand, Malaysia, Japan and Indonesia) as keynote speech and participants. 24 papers were selected to be included in this proceeding from 28 oral and 31 poster presentation.

This proceeding is contained of three sub chapter, that is fruits, vegetables and miscellaneous. There are 9 papers of fruits chapter, 12 papers of vegetables chapter and 3 papers of miscellaneous chapter. We wish to thank Sanjeet Kumar, Ph.D, Prof. Sobir, Prof Masayoshi Shigyo, Dr. Mohd Desa Haji Hassim, Parson Saradhuldhat, Ph.D for being keynote speech at this international seminar and all participants for very lively atmosphere during and after the seminar.

Bogor, May 2017

Editor

Dr. Darda Efendi
Dr. Awang Maharijaya

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Fruits

International Seminar on Tropical Horticulture 2016

Evaluation of Morphological and Cytological Character of F1 Diploid Hybrid Banana Sapon and *Musa acuminata* var. *tomentosa* (K.Sch) Nasution

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Abstract

Banana breeding by crossing is one way to get a new cultivar with a better quality. The aim of the development of diploid banana hybrid was to obtain new cultivars that can be used as a parent for the next hybridization that have a source of disease resistance genes. The result of the selection between Sapon banana with wild banana *Musa acuminata* var. *tomentosa* produced SNMT hybrid banana that had been characterized by morphology and cytology. In morphology, SNMT hybrid had characters like the female parent Sapon banana and produced seeds. The test using flowcytometry showed the ploidy level of SNMT hybrid was diploid. The cytological validation showed the chromosome number of SNMT hybrid 2n = 2x = 22. The diploid SNMT hybrid banana could be a source of potential genes in the next banana breeding.

Keywords: Banana breeding, diploid SNMT hybrid, Sapon banana, *Musa acuminata* var. *tomentosa* (K.Sch) Nasution

1. Introduction

Indonesia is one of the center of banana genetic diversity in the world, both cultivated and wild. The development of disease-resistant banana varieties, conventionally were inhibited by long time generation, triploid, parthenocarpy and sterility of the most edible cultivars. Commercial bananas could be produced from triploid banana cultivars. Nevertheless, diploid banana is very important as a source of allele for resistance or tolerance to biotic and abiotic factors (Jenny *et al.* 1999).

In banana breeding, the character informations of morphology, agronomy, physic and chemical were very useful in the selection of parents for cross breeding and selection for the development of diploid improved banana. This development can be used as a source of crossing between diploid improved and triploid to obtain tetraploid hybrid plants. The important agronomic traits are the shorter plants, resistance to the disease and better fruit quality (Silva *et al.* 2002).

The estimation of the genome size and the ploidy level of the putative hybrid is the first step in the breeding evaluation. Two methods that commonly used are calculating the number of chromosomes and analyzing by flowcytometry. Flowcytometry is a technique of quantification genome, originally developed for research in biomedical and adapted for the analysis of plant genetics (Segura *et al.* 2007). This technique gives accurate method to estimate the ploidy level

by calculating the proportion of cells in phase G1, S and G2/M of the cell cycle (Dolezel *et al.* 1989). This data also can be used to calculate the time of the cell cycle, which is used in genetic studies and useful for the analysis of growth and development (Loureiro *et al.* 2007).

In this study, we performed a crossing between cultivated banana Sapon/wild banana *M. acuminata* var. *tomentosa*. The results of this crossing can be used as a parent later to obtain new varieties of bananas that have good characters. This study aimed to evaluate the morphological and cytological banana hybrid diploid SNMT that can be used as a parent for banana breeding programs.

2. Methods

Sapon banana, wild banana *Musa acuminata* var. *tomentosa* and their hybrid had been planted in Banana Germplasm Gardens, Research Center for Biology, Indonesian Institute of Sciences. A cross between Sapon banana and *M. acuminata* var. *tomentosa* was conducted in August 2012. Pollination was conducted by taking pollen from wild banana *Musa acuminata* var. *tomentosa* and put it on female flowers of Sapon. Pollination was done in the morning between at 8-10 am. After pollination, the bunches of flowers were covered with a fine mesh (insect net) to prevent a cross-pollination by insects or other pollinators. Fruit of hybrids was harvested at physiological maturity.The hybrid seeds were separated from flesh of the full ripe and soft fruit. It was seeded by in vitro embryo rescue method. Planting in the field was done with 3 m x 3 m of space.

Characterization of banana hybrid diploid was made by 52 characters (UPOV, 2010) and 15 important characters in Descriptors of Musa (IPGRI, 1996). Qualitative and quantitative characters were measured and observed at the time of flowering, before harvesting, at harvesting and post-harvesting.

Ploidy level observation was done by a flowcytometry protocol that have been developed for bananas (Dolezel *et al.* 2004). The average of DNA content (mean) and the coefficient of variation (CV) of each sample at each peak was observed and compared with control plants, and was determined in accordance with the ploidy level of average number of DNA content. Ploidy level validation was also performed by observing the number of chromosomes. This protocol was conducted based on Adeleke *et al*. (2002) with modification. We used the meristematic part of the male flower. Male flower was split longitudinally in half, then took the youngest section like a cone. Immediatelly, it was soak in the Clarke solution 3:1 ethanol: acetic acid for 30 to 60 minutes. Then several brachtea were peeled until the size of male flower 0.5-1 cm and rinse in sterile distilled water three times. Took about 10- 15 anthers and put into 2 ml tube containing 200 µl of enzyme mixture solution (1% cellulase, 1% pectyolase, 1% cytoglicase dissolved in 10 mM citrate buffer). Then, incubated in the hotplate at 37° C for 2 hours. After that, the tube was put in ice and rinse with sterile distilled water 3 times. Then 1 ml solution of 3: 1 methanol: acetic acid was added and it can be stored in the freezer until it will be used. For slide preparation, one anther was taken placed on the object glass, added 1 drop of a solution of 3: 1 methanol: acetic acid. It was pressed gently using needles until the pollen out and spread. the other tissue that was not needed was separated. One drop of a solution of 3: 1 methanol: acetic acid was added, and heated over the fire and dried briefly at room temperature. After that, 15 µl dye solution DAPI (4',6-diamidino-2-phenylindole): vectashield (1:20) was added and covered with a cover glass. Chromosome counting was done by using Fluorescent microscope (Olympus type BX53) with 1000 X magnification with immersion oil.

3. Result and disscussion

The performance of quantitative and qualitative characters of hybrid diploid SNMT had moderately spreading habitus. Its habitus like the female parent Sapon banana. The pseudo-stem was light green and yellow-green inside. The leaves were green with a yellow midrib and no glossiness on the top of the leaves. The male influorescent shape was lanceolate, similar to the banana Sapon. The male parent wild banana *Musa acuminata* var. *tomentosa* was contained in hybrid diploid SNMT, i.e, the shape of the base of the leaf blade was one side rounded and one side acute. The color of inner barchtea was red and the shape of brachtea tip was right angle. The hybrid SNMT seeds were conducted by in vitro embrio rescue (Table 1).

Table 1. Qualitative characters of hybrid SNMT banana (Sapon X *M. acuminata* var. *tomentosa*)

Characters	Sapon	*M.acuminata* var. *tomentosa*	SNMT
Ploidy	2x=22	2x=22	2x=22
Growth habit	Spreading	Upright	Spreading
Color of pseudostem	Light green	Green	Light green
Color of inner pseudostem	Light green	Yellowish green	Yellowish green
Compactness of crown	Medium	Medium	Loose
Attitude of wing at base of petiole	Curved outwards	Straight	Curved outwards
Color of midrib on lower side	Yellow	Yellow	Yellow
Shape of base of leaf blade	Both side acute	One side rounded one side acute	One side rounded one side acute
Waxiness on lower side of leaf blade	Absent	Absent	Weak
Glossiness of upper side of leaf blade	Absent	Absent	Absent
Pubescence of peduncle	Present	Present	Present
Shape of bunch	Cylindris	Irregular	Irregular
Attitude of fruit of bunch	Moderately turn up	Moderately turn up	Horizontal to slightly turn up
Compactness of bunch	Loose	Medium	Medium
Attitude of male inflorescence	Vertikal	Inclined	Horizontal with inclined end
Prominence of scars	Weak	Weak	Weak
Persistence of bracts	Weak	Weak	Weak
Persistence of hermaphrodit flower	Absent	Absent	Absent
Longitudinal curvature of fruits	Slightly curved in distal part	Evenly curved	Slightly curved in distal part
Color of peel before maturity	Green	Green	Green
Persistence of floral organ	Absent	Absent	Present
Color of flesh of fruits	Yellow	Yellow	Yellow
Firmness of flesh	Soft	Soft	Soft
Seeds	Absent	Many	Few
Persistence of male inflorescence	Present	Present	Present
Shape of male inflorescence	Lanceolate	Narrow ovate	Lanceolate
Overlap of bracts	Weak	Weak	Medium
Color of inner bracts	Red orange	Red	Red
Shape of apex of bracts	Broad acute	Right angle	Right angle

Banana breeding program aims to develop resistant varieties to pests and diseases. The strategy is focused on agronomic aspects of the crop, organoleptic character, tolerance to stress,

shelf life, mineral content, application of water and resistance to damage mechanism (Bakry *et al.* 2008).

Quantitative characters of hybrid diploid SNMT bananas showed that the height and diameter of the pseudostem, the number and lenght of leaves, the number of suckers, the length of petiole relatively were greater than the female parent. However, the size of fruit including weight, length, and diameter of fruit, length and thickness of fruit pedicellus were smaller than the female parent (Table 2).

Table 2. Quantitative characters of hybrid diploid SNMT (Sapon X *M. acuminata* var. *tomentosa*)

Characters	Sapon		*M. acuminata* var. *tomentosa*		SNMT	
	Mean		Mean		Mean	
Height of pseudostem (cm)	172.5	±10.60	252.67	±24.79	221.6	±44.77
Diameter of pseudostem (cm)	7.95	±1.35	9.93	±1.29	12.64	±2.99
Number of leaves	4	±0.00	9.33	±1.15	6.6	±2.07
Number of suckers	1.5	±0.70	16	±6.56	4.8	±1.48
Lenght of leaf blade (cm)	124	±5.65	128.33	±20.60	207.2	±9.73
Lenght of petioles (cm)	23.5	±6.36	73.67	±13.61	44	±7.62
Widht of leaf blade(cm)	38	±4.24	30.33	±4.51	62.9	±2.84
Weight of fruit (g)	77.48	±13.28	28.84	±3.3	26.20	±8.72
Length of fruit (cm)	17.84	±2.03	9.00	±1.59	10.8	±2.48
Diameter of fruit (cm)	2.86	±0.21	2.32	±0.15	2.28	±0.43
Lenght of pedicellus (cm)	1.50	±0.68	0.78	±0.22	1.49	±0.59
Thickness of peel (cm)	0.26	±0.03	0.14	±0.03	0.16	±0.06
Brix	12.00	±1.8	12.6	±3.75	9.4	±2.26
Ph	4.38	±0.3	4.85	±0.39	4.84	±0.29

The presence of the seeds of hybrid showed the fertilization of pollen of male parent with ovaries of the female parent. According to Poerba *et al* (2016), the factors that affect the formation of seeds from crossing involve the genotypes and environmental conditions. It also occurs as a result of variations in the pollen of male parent in forming the pollen tube. Instance, the pollen of rejang banana that not all of them can form the normal pollen tube so that the seed formation is very low.

Wild banana *Musa acuminata* produces many seeds resulted from an open-pollination. The most of seeds have a normal embryo, where a small number of abnormal seeds showed no embryo/endosperm or both. If more than 90% of the seeds can germinate, it can be used to study the hybrid populations for breeding of banana. Pollination in banana is cross-pollinated, and it would be interesting to know the effects of resistance and seed germination on inbreeding (Javed *et al.* 2002).

Figure 1. Performance of banana: (a) female parent Sapon banana (2x), (b) male parent wild banana *Musa acuminata* var. *tomentosa* (2x), (c) SNMT hybrid diploid (2x); bar= 5 cm

Identification of ploidy level of hybrid SNMT was conducted using flowcytometry. The quality of nucleus suspension cell can be seen from the histogram analysis of relative nuclear DNA content. Debris on the histogram should be as minimal as possible. The peak of G1 (G2) to be symmetric and low variation. Variations are usually seen in the coefficient of variation (CV) = standard deviation/average peak x 100%. To estimate the amount of nuclear DNA, suspension or cell permeable nucleus stained with fluorochrome specific to DNA and the amount of light emitted by each nucleus could be quantified. The results of the analysis were displayed in the form of relative fluorescence intensity histogram, represents the relative DNA content (Dolezel and Bartos, 2005). From twelve hybrid SNMT banana observed, all of hybrids SNMT diploid banana had an average channel peak of ploidy level ranged from 211.39 to 247.84 by the coefficient of variation (CV) between 6.38 to 10.13 (Table 3).

Tabel 3. Measurement of ploidy level of hibrid diploid SNMT and its parent using flowcytometry.

No	No of accession	parent/ hybrid code	Mean	CV (%)	ploidi
Sapon banana diploid (female)					
1	I 21A#1 (a)	SN	210.64	8.11	2x
2	I 21A#1 (b)	SN	192.40	8.74	2x
***Musa acuminata* var. *tomentosa* diploid wild (male)**					
1	IV 3E#2	MT	170.86	9	2x
Sapon x *Musa acuminata* var. *tomentosa* (hybrid)					
1	VII 9H#1	SNMT	211.39	7.34	2x
2	VII 9H#3	SNMT	235.5	7.12	2x
3	VII 9H#5	SNMT	246.38	6.6	2x
4	VII 9G#1	SNMT	232.59	6.55	2x
5	VII 9G#2	SNMT	215.94	6.38	2x
6	VII 9G#4	SNMT	247.84	7.45	2x
7	VII 9G#5	SNMT	218.94	7.72	2x
8	VII 6A#1	SNMT	226.56	9.53	2x
9	VII 6A#2	SNMT	215.88	9.88	2x
10	VII 6A#3	SNMT	222.6	9.47	2x
11	VII 6A#4	SNMT	219.48	10.13	2x
12	VII 6A#5	SNMT	212.58	9.8	2x

In addition, to determine the number of chromosomes in banana, it could be used cytological examination. Usually, the root tip was used, but it needs more long time and requires special skills to make good slides. The male inflorescence could be used to determine the number of chromosomes. The male flower part that used was the youngest (meristematic) and in the innermost layer. Anther size was used range between 0.5-1 cm. By using anther, we can see the pollen mother cells could be observed and the number of haploid chromosomes and the companion cells that are apart of the somatic cells could be determined. In this study, the SNMT hybrid banana has the chromosome number of 2x = 22, similar to that held by using two parents (Figure 2).

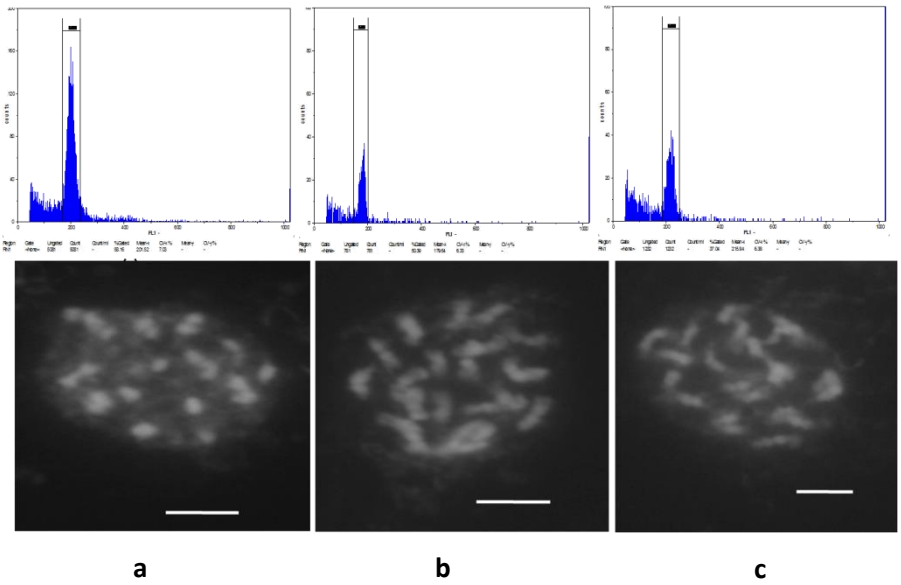

Figure 2. The chromosome number and ploidy level of SNMT hybrid diploid and its parents. (a). sapon banana, (b) wild banana *Musa acuminata* var. *tomentosa*, (c) SNMT hybrid, bar = 10 µm.

4. Conclusion

Banana breeding programs by crossing produced a hybrid that has the characteristics and properties that are different from the parent. SNMT diploid hybrid is a hybrid between Sapon banana with wild banana *Musa acuminata* var. *tomentosa*. In morphology, SNMT hybrid had characters like the female parent Sapon banana and produced seeds. The test using flowcytometry showed the ploidy level of SNMT hybrid was diploid. The cytological validation showed the chromosome number of SNMT hybrid was 2n = 2x = 22. SNMT hybrid diploid is a new cultivar that can be used as a parent in the next banana breeding programs.

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Fruit Plants of Kalimantan : Results of Field Exploration and Conservation

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Abstract

Kalimantan is rich in its diversity of endemic fruit species, which include among others *Durio spp., Artocarpus spp., Sarcotheca spp., Nephelium spp., Euphoria spp.* and *Lansium spp.* on Central, South and West Kalimantan. The aim of this study was to inventory the potential of indigenous fruit plants in the province of South, Central and West Kalimantan and *ex situ* conservation in the Kalimantan Botanical Gardens. This was done by field exploration of indigeneous fruit plants and *ex situ* conservation efforts in Banua Botanical Garden (South Kalimantan), Katingan BG (Central Kalimantan) and Sambas BG (West Kalimantan). Based on these observations, There are five species of most dominant of the results of field exploration, namely *Durio* spp (Malvaceae), *Nephelium* spp. (Sapindaceae), *Mangifera* spp. (Anacardiaceae), *Musa* spp. (Musaceae) and *Artocarpus* spp. (Moraceae). However five families were dominant at three Botanical Gardens, i.e. Myrtaceae (27 specimens), then Sapindaceae (24 specs), Malvaceae (20 specs), Anacardiaceae (13 specs) and Moraceae (12 specs).

Keywords: *Durio spp.*, fruits, Kalimantan, *ex situ* conservation, Banua Botanlcal Garden, Katingan BG, Sambas BG.

1. Introduction

Kalimantan is rich with high endemism of the flora, about 34% of Indonesian wild plants are endemic to Borneo (Ashton, 1982). However, human activities such as development of human settlement and plantation, lead to the decrease of forest area, gradually. One of the potential economic value of forest, that forest fruit trees are diverse in Kalimantan. However, the potential fruits remain undeveloped. This is caused partly because of the standpoint of forestry, forest fruits still considered as forest products (minor forest products) and are considered less important economically (Prosea 1991; Prosea 1993). Whereas Kalimantan beside the famous richness and diversity of germplasm of local fruits, the area is also a center of distribution of some commodities tropical fruits of high economic value (Uji 2004). But unfortunately richness and diversity of germplasm of indigenous fruits are abundant enough is apparently not fully utilized. In addition, it also would be much better if a region chooses to utilize and develop the indigenous plants in the area because it naturally has adapted well. Therefore, research on species diversity and richness of germplasm of indigenous fruits of Kalimantan needs to be done. There are 226 species of indigenous fruits encountered in Borneo (Uji, 2004). From reviews these records, fifty-eight species are cultivated, and 31 species are as endemic.

Local community forest planting various species of plants that are useful include forest fruits in orchards. They call this garden location as lembo, munan, simpuk, Pulong bua, bua Dalung, tundang kemurlan, Kanoka kemurlan, tembawang and hamlets (Siregar, 2006). Other activities that can support the preservation efforts of fruits indigeneous to Indonesia, among others germplasm with established gardens, botanical garden, arboretum gardens, and others. Therefore, to protect forest areas from the threat of land conversion, the need for regional

protection measures protected forests, forests and nature in situ conservation or habitats that exist naturally (Nature Reserves and National Parks). But the condition of the area that has increasingly pressured so needed another area which is an area of *ex situ* conservation or artificial habitat in order to protect the plant in the Botanical Gardens, such as Banua Botanical Garden, Regency of Banjarbaru, South Kalimantan Province; Katingan Botanical Garden, Regency of Katingan, Central Kalimantan and Sambas Botanical Garden, Regency of Sambas, West Kalimantan Province. Referring to the Presidential Decree No. 93 of 2011 on the Botanical Gardens, the botanical garden functions include: conservation, research, education, tourism, and environmental services.

The purpose of this study to inventory the potential of indigenous fruit plants in the province of South, Central and West Kalimantan and *ex situ* conservation in the Kalimantan Botanical Gardens.

2. Methodology

Location tread Banua Botanical Garden, Regency of Banjarbaru, South Kalimantan Province; Sambas Botanical Gardens, Regency of Sambas, West Kalimantan Province; and Katingan Botanical Gardens, Regency of Katingan, Central Kalimantan (Figure 1). The samples was collected in around of South Kalimantan province, West Kalimantan province and Central Kalimantan province. Katingan Botanical Garden located adjacent to the tourist area in Bukit Batu, Village Kasongan Lama, District Katingan Hilir, Katingan, Central Kalimantan Province. Land area of 127 ha with an altitude 40-70 m above sea level (asl.). Geographically located at coordinates S 01°0.53'37.2"- E 113°28'05". Sambas Botanical Garden is located in the Sambas Land Use Areas (APL) and is administratively located in the village Sabung, District Subah, Sambas regency. Sambas Botanical Garden that extent ± 300 Ha in coordinates E 109º 27'47.05"- 109º29'24.14" and N 01º15'45.22"- 01º17'3.30" with an altitude 32-75 meter above sea level (asl). Research for Banua Botanical Garden conducted on 19 March to 7 April 2014 (1) Natural lnhutani II sea Island, Kota Baru; (2) Forest Nature of Swangi islands, Batulicin; and (3) Tahura Sultan Adam, Mandi Angin, Banjar at an altitude of 75-300 m above sea level. The study was conducted in 2010, 2013 and 2014.

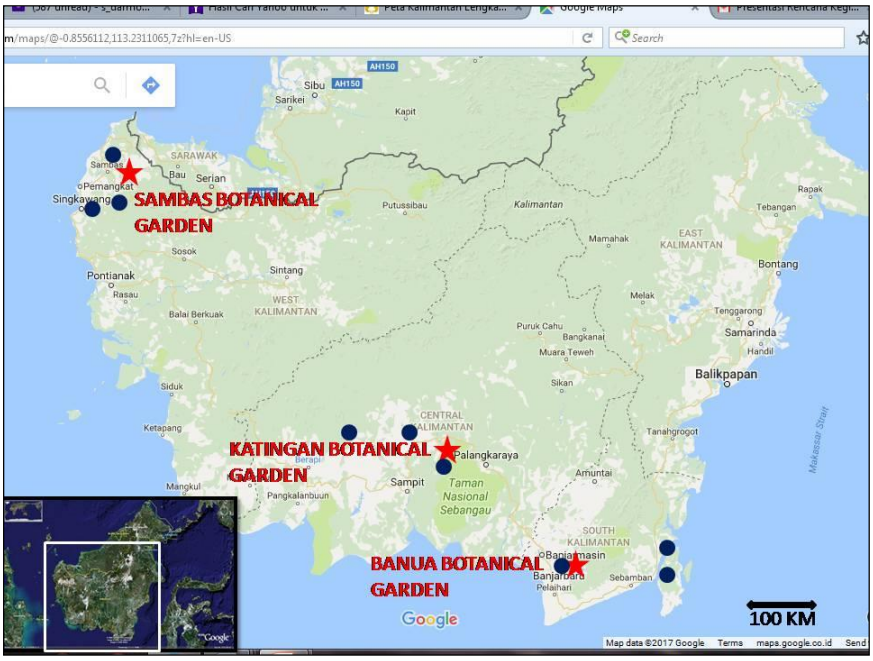


Figure 1. Map of research site at Kalimantan. Three botanical gardens (star) and samples sited for each around botanical garden which is as living collection (circle).

Documenting plants collected in this research activity is recorded on a "field book" that has been prepared in a standard by Bogor Botanical Gardens. The material collected is recorded identity, habitat and morphology of data and if there is information ethnobotany of local residents or officials of officers from the Regional Agencies that accompany the exploration activities as well as from local guides at these locations. In this study restrictions on the species of fruit that can be eaten (edible fruits) are covering all species of annual plants that produce edible fresh fruit and either cooked or raw fruit that is found by the research team. Fruits also include either a major functions or minor functions (Prosea 1991; Prosea 1993). For species of fruits term "indigenous of Indonesia" is the kind of local fruits that grow naturally or originating from the area of Indonesia (Uji, 2004). Species of fruits are hard-skinned, not broken and seed in one or the so-called "nut" is not included in this article. Data agroecological include measurements of pH, temperature, humidity, soil moisture, altitude, and coordinates (longitude and latitude) using GPS. All data written on Form A1 which will be equipped with an access number from the Author (MN) as standard accessing code at Bogor Botanical Gardens. The data obtained during the study comes with documentation in the form of photographs for material life, fruit, and activities in the field.

3. Results and Discussions

A. Results

At figure 2, shows of all exploration results in the three provinces, that 71% of them of the indigeneou fruit plants of Kalimantan has collected as living collections at the Katingan Botanical Garden. While some are still a little collected as living collections at Banua Botanical Garden (South Kalimantan; 15%) and Sambas Botanical Garden (West Kalimantan: 14%). This condition is still bearish as the growing conditions still takes a long time, because the fruit plants will naturally be able to bear fruit after 7-10 years. Botanical Garden as an institution is expected not to convert so that the calculation of the age of the fruit plant collections will still continue to be listed in the full document, which is the time of planting, flowering, fruiting and death. In addition to the current research in Central Kalimantan is already obtain location information of indigeneous fruit plants where its existed.

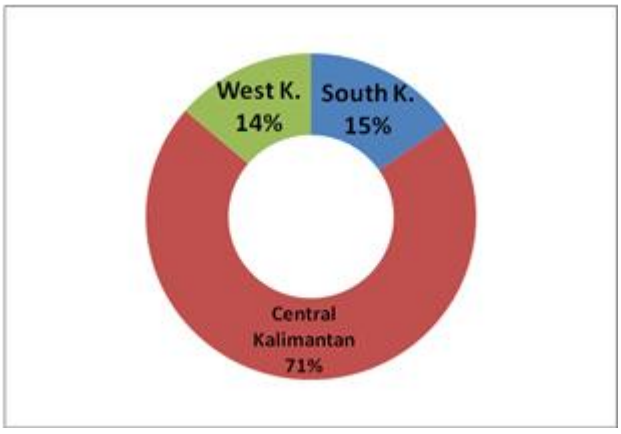


Figure 2. The percentages of indigenous fruit collected at three botanical garden (Banua Botanical Garden, Regency of Banjarbaru, South Kalimantan Province; Sambas Botanical Gardens, Regency of Sambas, West Kalimantan Province; and Katingan Botanical Gardens, Regency of Katingan, Central Kalimantan)

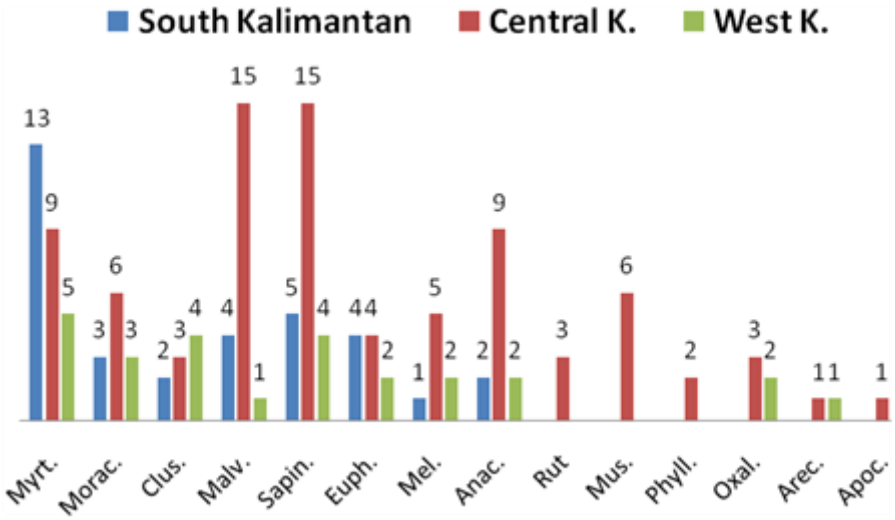


Figure 3. The number of specimens existing of exploration results on the three botanical gardens in Kalimantan

In figure 3, most of indigenous plants of fruit trees existing in Central Kalimantan. Family that dominantly, i.e. Malvaceae (15 specimens) and Sapindaceae (15 specs), while the other family Anacardiaceae (9 specs), Musaceae (6 specs), Moraceae (6 specs), Meliaceae (5 specs), Euphorbiaceae (4 specs) Rutaceae (3 specs), Oxalidaceae (3 specs), Clusiaceae (3 specs), Phyllanthaceae (2 specs), Arecaceae (1 spec) and Apocynaceae (1 spec). At the family Malvaceae, *Durio* spp majority as genus and the family Sapindaceae by genus *Nephelium* spp. As for the other family, the Anacardiaceae (*Mangifera* spp.), Musaceae (*Musa* spp), Moraceae (*Artocarpus* spp). Figure 3 shows the location in South Kalimantan is dominated by family Myrtaceae (13 specs) and Sapindaceae (5 specs). While other family Malvaceae (4 specs) and Euphorbiaceae (4 specs). Genus of family Myrtaceae, *Syzygium* spp dominantly, but it is unclear that identification of the species name until look of flowers and fruits. At the family Sapindaceae diverse as there are genera *Nephelium*, *Dimocarpus, Pometia* that still need to be investigated further as a new species or new variety. At the location of West Kalimantan specimens obtained less than the Central and South Kalimantan. Family that dominantly, i.e. Myrtaceae (5 specs), Clusiaceae (4 specs), Sapindaceae (4 specs) and Moraceae (3 specs). While Euphorbiaceae (2 specs), Meliaceae (2 specs), Oxalidaceae (2 specs), Anacardiaceae (2 specs), Malvaceae (1 spec) and Arecaceae (1 spec), is not even found Rutaceae, Musaceae, Phyllanthaceae and Apocynaceae as on site of other Kalimantan (South and Central Kalimantan).

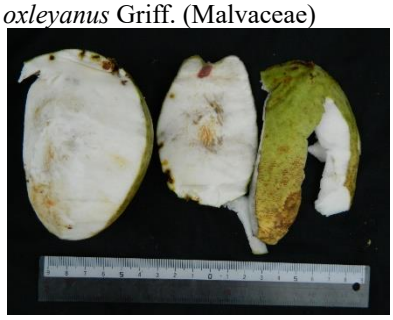
Dimocarpus longan Lour. (Sapindaceae)

Dimocarpus longan Lour. (Sapindaceae)

Durio dulcis Becc. (left), *D. kutejensis* (Hassk.) Becc., *D. oxleyanus* Griff. (Malvaceae)

Garcinia xanthochymus Hook.f. ex T.Anderson (Clusiaceae)

Lepisanthes amoena (Hassk.) Leenh. (Sapindaceae)



Mangifera caesia Jack. (Anacardiaceae)

Seedling and seed transport

Nursery of botanical garden

Planting at Botanical Garden

Figure 4. The species of indigenous fruit plants of Kalimantan and images of ex situ conservation in Banua Botanical Garden.

4. Discussions

Uji (2004) have also reported that there are 226 species of indigeneous fruits of Kalimantan which can be eaten either directly or after processing and are useful as a source of germplasm fruits. Siregar (2006) also reported that in Kalimantan there are 130 species of local fruit trees (both species of indigeneous and introduced) had been consumed by the local community. In Figure 3, on all locations in the three provinces there are some families that the number of specimens large enough, among others family Myrtaceae (27 specimens) and Sapindaceae (24 specs). Also the other family were also quite a lot, which Malvaceae (20 spec), Anacardiaceae (13 spec) and Moraceae (12 spec). These five families who have a diversity of fruits that have high potential to be studied and developed, because of high species diversity is the main capital in conducting the business of plant breeding. At viewed from the prospect of economic value, there are three families of the three genera that have good prospects to be developed in the future. All three genera according Winarno (2000) are *Garcinia* (Clusiaceae), *Mangifera* (Anacardiaceae), and *Nephelium* (Sapindaceae). In addition, it is also found one another family of the genus Malvaceae, *Durio* spp. is also potential to be developed (Figure 4). Four species of fruits commodity of the four genera has been defined as "fruits national champions", respectively, are mango, mangosteen, rambutan and durian (Winarno 2000). Mogea, *et al.*

(2001), recorded 62 species have been cultivated, 18 species are endemic and four species including rare plants. These four species of rare plants is kerantungan (*Durio oxleyanus*), lahong (*Durio dulcis*), lai (*Durio kutejensis*) and burahol (*Stelechocarpus burahol*). No fewer than 329 species of fruits (consisting of 61 families and genera 148) both of which are indigeneous to Indonesia and the species of introduced can be found in Indonesia (Rifai, 1986). In the Southeast Asian region reported there are about 400 species of fruits that edible (Prosea, 1991). Thus more than three-quarters of the species of fruits that were reported in the Southeast Asian region has been found in Indonesia (Purnomo *et al.* 2002). Based on the results of the data collected was recorded 266 species of fruits indigeneous to Indonesia has been found that most still grows wild in the forests and only a small portion that has been cultivated (Uji 2007). With the percentage of the number of tree species that most (76%) it demonstrates that for fruits plant breeding efforts required considerable time because of the long life cycle of tree species (Mogea, *et al.*, 2001).

Existing of Botanical Gardens in Kalimantan, there are four Regional Botanic Gardens, i.e. Katingan Botanical Gardens (Central Kalimantan), Banua Botanical Garden (South Kalimantan) and Sambas Botanical Garden (West Kalimantan), in fact there are also in East Kalimantan (Balikpapan Botanical Garden). Botanical Gardens is a representation of *ex situ* conservation in areas that have a high diversity of plants (Figure 4). According Uji (2004), based on the location of the number of species most commonly found in Sumatra (148 species) and Kalimantan (144 species), next is the Java (96 species), Sulawesi (43 species), Maluku (30 species), Nusa Tenggara (21 species), Papua (16 species) and 34 other species spread throughout Indonesia. Kalimantan and Papua are still small when compared to five other areas. This is partly due to data on the flora of both, especially data on fruit plants is still not widely known and reported. Therefore the *ex situ* conservation in botanical gardens should more built in Kalimantan so that the current indigeneous fruits plants of Kalimantan where still grown in the yard by community collected immediate as much as possible.

5. Conclusion

The study of fruit plants were conducted in three provinces in Kalimantan, the overall earned 71% of its collected as living collections at Katingan Botanical Garden, 15% in Banua Botanical Garden (South Kalimantan) and 14% in Sambas Botanical Garden (West Kalimantan). Family Myrtaceae at three Botanical Gardens at most (27 specimens), then Sapindaceae (24 specs), Malvaceae (20 specs), Anacardiaceae (13 specs) and Moraceae (12 specs). *Durio* spp (Malvaceae) is the dominant genera, then *Nephelium* spp. (Sapindaceae), *Mangifera* spp. (Anacardiaceae), *Musa* spp. (Musaceae) and *Artocarpus* spp. (Moraceae). Attempts to plant breeding of fruits takes a long time and Botanical Gardens in Kalimantan is expected to protect the parents of indigeneous germplasm fruit plants to the area.

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Melon Breeding: Past Experiences and Future Challenges

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Abstract

Melon (*Cucumis melo* L.) is a species with the largest genetic diversity among others in the Cucurbitaceae family, and therefore providing opportunities for plant breeders to develop new, improved varieties. C. *melo* var reticulatus (North American cantaloupes), C. *melo* var inodorus (honeydews), and C. *melo* var cantalupensis (European cantaloupes) are the most widely known among at least eight cultivar groups. Melon is a cross pollinated species where most of pollination events are performed by bees. F_1 hybrid is the most common type of varieties could be found in the market today. Some important fruit traits in melon include: fruit weight, sugar content, flesh color and texture, rind appearance and hardness, and shelf-life. An ideotype of large fruit with an attracting orange or green, sweet and crisp flesh are more demanded nowadays for Indonesian market. Additionally, obtaining varieties resistant to main pests and diseases are of importance as well. We have been conducting a melon breeding program aimed for quality fruits at the Center for Tropical Horticulture Studies, Bogor Agricultural University (IPB), and two honeydew varieties has been released. Recetly we identified a melon genotype (IPB Meta 9) exhibiting good resistance to downy mildew and can be utilized in a breeding program. Future challenges include shifts in consumer preferences, for example, small-size fruits may be more preferred for personal or small family consumptions.

Keywords: breeding, fruit quality, melon

1. Genetic diversity of melon

Melon (*Cucumis melo* L.) is a cross-pollinated diploid species (2n=2x=24) with the largest genetic diversity among other species in the Cucurbitaceae family (Dutt and Saran, 1994; Nayar and Singh, 1994). Wild species of *Cucumis* occur in Africa. Secondary centers of diversity include Persia, Southern Russia, Iran, Afghanistan, India, and China. Cucumber (*Cucumis sativus* L.) is known as a close relative to melon based on combined chloroplast and nuclear data (Renner *et al.* 2007). The C. *melo* species has at least eight cultivar groups or botanical varieties, i.e. *C. melo* var. reticulatus (North American cantaloupe or muskmelon), *C. melo* var. inodorus (honeydew), *C. melo* var. cantalupensis (European cantaloupe), *C. melo* var. makuwa (oriental melon), *C. melo* var. flexosus (snake melon), *C. melo* var. conomon, *C. melo* var. chito, and *C. melo* var. dudaim (Robinson and Decker-Walters, 1999). Reticulatus, inodorus, and cantalupensis are the most widely known cultivar groups in many countries including in Indonesia. Reticulatus melons typically have thick, netted rind, moderate shelf-life, green or orange thick flesh with firm texture, and mature fruit slips from the stem. Inodorus melon generally have smooth (non-netted) rind, long shelf-life, flesh color of white, green, or orange, crisp flesh texture, and mature fruits does not slip from the stem. Catalupensis melons typically have strong aroma, short shelf-life, juicy flesh, and vertical sections in the rind.

Our previous study evaluating a diverse collection consisting of 30 melon genotypes (13 F_1 hybrid varieties, 4 F_2 populations, 6 open pollinated varieties, and 7 inbred lines) indicated a good opportunity for breeding new, improved varieties. These genotypes can be classified into the reticulatus (12 genotypes), inodorus (8 genotypes), and makuwa (3 genotype) groups; however we found some reticulatus genotypes with some cantalupensis group's characteristics (e.g. stronger aroma, shorter shelf-life, juicier flesh), and therefore we put those into the 'cantalupensis-like'group (7 genotypes). Fruit appearance of some of the genotypes are shown in Figure 1. F_1 hybrids selected based on fruit weight and sugar content are: Action, Autumn Favor, and Monami Red from the reticulatus group; Jade Flower from the inodorus group, and Hales' Best from the cantalupensis-like group.

Figure 1. Genetic diversity of fruit appearance among melon genotypes

2. Flower types and pollination

Most cantaloupes and honeydews cultivars are andromonocious, i.e. has both male and hermaphrodite flowers in one plant (Robinson and Decker-Walters, 1999). Male flowers appear on the main and secondary branches, whereas hermaphrodite flowers appear on the secondary or higher order branches. Monoecious cultivars (having male and female flowers in one plant) are less common, although are more desirable for hybrid seed production because emasculation (removing anthers) from the female plants would not be required. Monoecious is dominant to andromonocious and the difference among these are controlled by a pair of alleles (More and Seshadri, 1994).

Main pollinating agent for melon plants is honey bees and the outcrossing amount is 5 – 70%. Higher fruit set (98%) occured under natural pollination as compared to hand pollination

(68%) (Munshi and Alvarez, 2005). From a study of melon pollination in an area containing two cantaloupe genotypes, greater number of bee moves between the two strains were observed than that within the same strain (Foster and Levin, 1967).

Steps involved in making crosses among melon genotypes are described in Figure 2. Emasculation of hermaphrodite flowers of an andromonicous parent should be performed one day before anthesis. Both male and hermaphrodite flowers need to be covered. Hand pollination should be done in early morning (e.g. by about 6 AM in Bogor, Indonesia) to avoid undesireable pollen contaminations by bees.

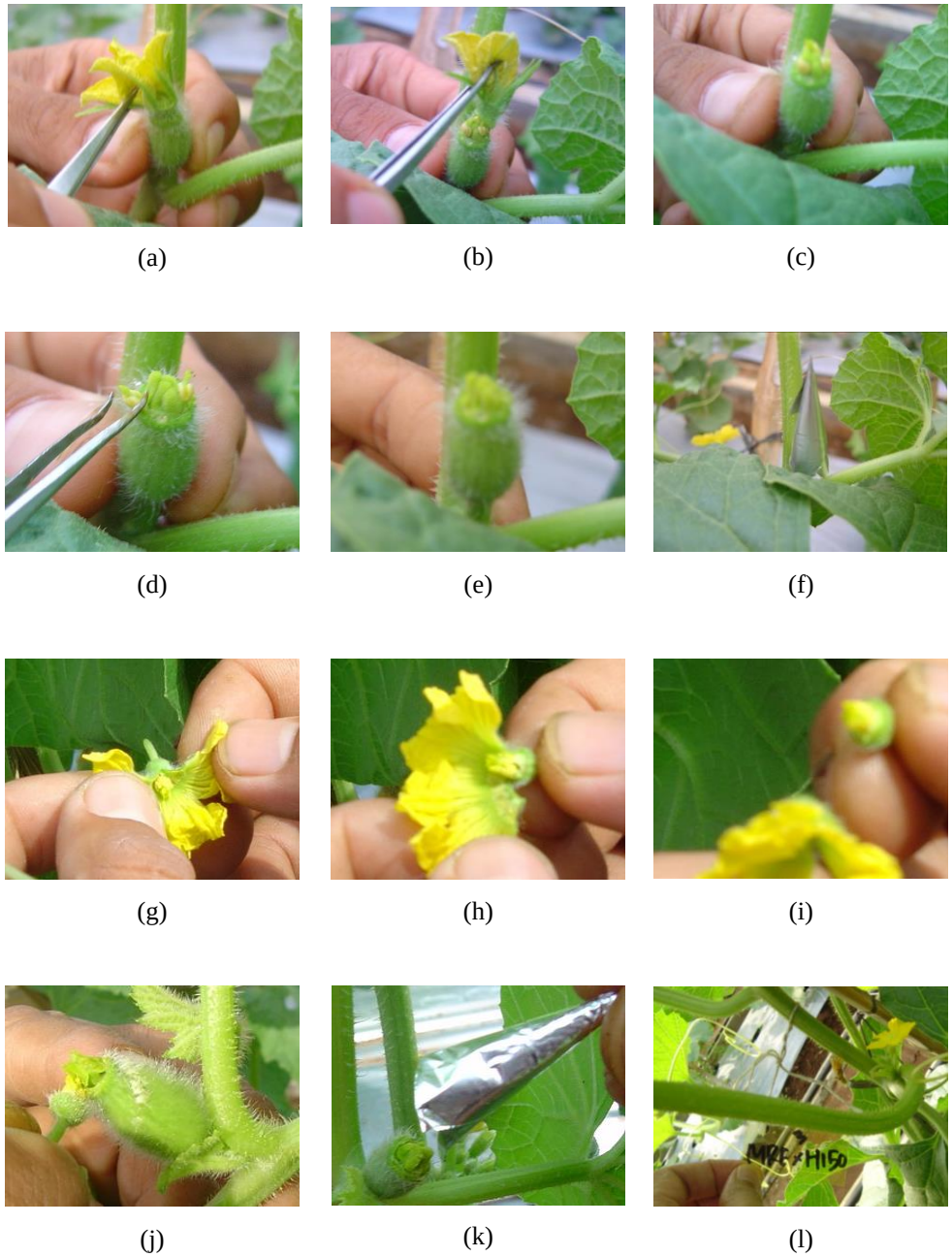

Figure 2. Steps involved in making a controlled crossing among melon genotypes: emasculation of a hermaphrodite flower on a female parent plant, before anthesis (a-f), pollination, performed the next morning (g-j), covering (k), and labelling (l).

3. Breeding objectives and methods

Ideally, breeding objectives are set to meet a melon ideotype demanded by consumers. The other less ideal practice is, the objectives may be set by the breeders and later the new released varieties will be advertized to the consumers. Fruit traits could be of most importance for consumers, and therefore the breeders should put considerable efforts into them. Some important fruit traits are: high sugar content, high yield, thick flesh, crisp flesh texture, attractive flesh color (usually green or orange), no unpleasant after-taste, hard rind for transportations, long shelf life, and good-looking dense net (for cantaloupes). No less important are breeding for pest and disease resistance. Important diseases in tropical regions include powdery mildew, downy mildew, bacterial wilt, and viruses. Additionally, breeding for adaptation to marginal environments, for example, drought tolerance, acid soil tolerance, or salinity tolerance could be of important considerations as well.

Because melon is a cross-pollinated species, its breeding programs could be targeted for developing improved open-pollinated varieties (OPVs) or hybrid varieties. Hybrid varieties are more common nowadays than OPVs eventhough their seed price are more expensive. Most melon hybrids available in the market are single crosses resulting from controlled mating of two inbred lines. Three-way cross and double cross varieties of melon hybrids are rarely available. An advantage of the single cross hybrid is theoretically more uniform than the other type of hybrids and the OPVs. Additionally, a hybrid melon variety may posess a specific combination of desirable traits (in our example, flesh and rind color) from its female and male parents. Characterizations of the traits may utilize a well developed descriptor such as from (IPGRI, 2003).

Breeding improved melon varieties involves four important practical steps: (1) development of base populations, (2) development of inbred lines, (3) generation of hybrids through controlled mating among inbred lines, (4) evaluation of the hybrids. A base population is a segregating popoulation that can be derived from a controlled mating among two genotypes (for example, see (Zuniga *et al.* 1999)), or from a topcross. Inbred lines can be developed from base populations following the pedigree breeding method (Robinson, 2000). In our example illustrated in Figure 3, we have two F_2 base populations named A and B. Manual self-pollinations (selfing) were conducted in selected plants from each populations, and the resulting fruit would contain S_1 seeds. The S_1 seeds from each fruit were kept separately and planted in groups in the following season. Selections were made among and within gorups, and the selected S_1 plants were selfed to produce S_2 seeds. In a practical breeding program for fruit traits, we selfed a number of plants and then perform selection based fruits performance later. These processes continue until at least S_7 generation. The S_7s or more advanced generations have homozygous genotypes in most of the loci, and hence can be considered as inbred lines. Genetic diversity within an inbred line theoretically are small, among lines derived from the same base population are larger, and among lines from different base populations are even larger. The hybrids, therefore, are suggested to be produced by crossing the lines derived from different base populations for obtaining aimed combinations of the traits as well as some amount of heterosis. Studies reported positive heterosis for fruit shape in melon (Fernandez-Silva *et al*. 2009; Jose *et al.* 2005).

Inbreeding depression are a phenomena typically observed during development of inbred lines of a cross pollinated species. We observed somewhat higher inbreeding depression level for fruit weight in reticulatus (netted) groups than in inodorus; whereas for sugar content, we did not notice a considerable level of such a depression.

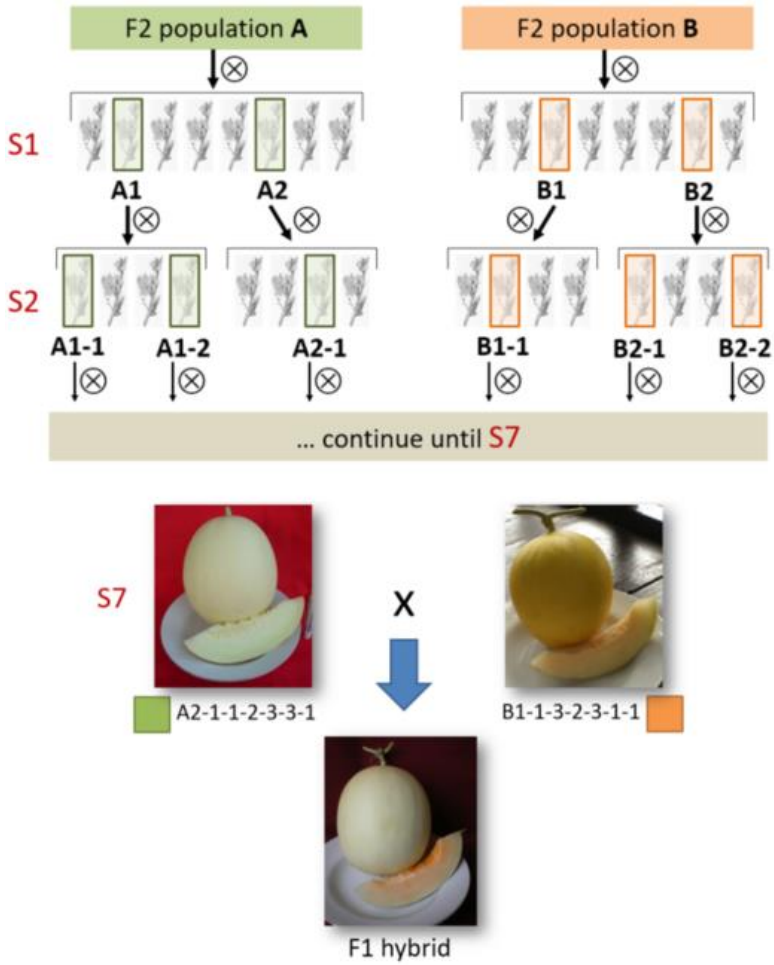

Figure 3. Illustration of steps involved in hybrids melon breeding

Our previous study evaluating 29 melon genotypes showed that fruit weight was positively correlated with fruit diameter ($r=0.96$, $P<0.01$), flesh thickness ($r=0.82$, $P<0.01$), fruit length ($r=0.67$, $P<0.01$), and rind thickness ($r=0.49$, $P<0.01$). We performed a path analysis for partitioning the correlation coefficients into direct and indrect effects, using fruit weight as an independent variable (Y), and stem diameter, days to harvest, fruit length, fruit perimeter, rind thickness, and flesh thickness as dependent variables (X). The direct effects of fruit perimeter on fruit weight were positive and large (0.83), while the indirect effects through the other X's are negligible. Altogether, these six X traits could explain 96% of the total variability of fruit weight.

4. Future challenges

The demand for melon fruits implies the necessity of quality seeds production of improved varieties. Changes on consumer perferences need to be anticipated through development of new, unique varieties with excellent tasting quality. Small-size melon cultivars will perhaps be popular for small family or personal consumptions.

Planting melon in tropical regions faces the challenges on dealing with pest and diseases. Downy mildews and viruses, among other diseases, are regarded as very important melon diseases leading to considerable economic loses, and hence breeding melon varieties resistant to these diseases could be among the top priorities. The 'IPB Meta 9' melon genotype from our breeding program showed moderate resistance to downy mildew (Huda and Suwarno, 2016). Its fruits, however, are small (less than 500 g on average) with thin and sweet white flesh. This implies that subsequent breeding activities are needed to introduce the resistance to another genotypes, and/or to 'fix' some undesireable characteristics posessed by this genotypes.

Our breeding experience suggested that breeding reticulatus (cantaloupe; netted) melons are more challenging somewhat than breeding inodorus (honeydew; non-netted) melons in terms of achieving uniformity of the lines. The cantaloupes unfortunately are still more popular than the honeydews in Indonesia, and therefore varieties from both groups need to be bred still. Large-size cantaloupes with sweet, orange thick flesh, and strong rind are on market demand at present and also in near future, we predict. Breeding honeydews could be aimed for obtaining fruits with thick, sweet and crisp flesh, along with unique rind appearance. Additionally, breeding for nutritions could also be an interesting option as melon is a good source of vitamin C. We recently observed a considerable range of genetic diversity for vitamin C concentrations among several melon genotypes in our breeding program.

Finding genes controlling a trait of interest is simply challenging; however genotyping tools are becoming more available recently. Marker-assisted selection could be an option for accelerating the breeding process, especially for traits that are controlled by single or few genes. Gene-finding approaches such as QTL or associaton mapping requires both genotypic and phenotypic data. Several recent studies have been conducted for studying QTL controlling important traits in melon, for example fruit traits (Monforte *et al.* 2004; Ramamurthy and Waters, 2015; Wang *et al.* 2016), fruit ripening and fruit softening (Moreno *et al*. 2008, Vegas *et al*. 2013), yield-related traits (Zalapa *et al.* 2007), powdery mildew resistance (Fukino et al. 2008; Wang *et al.* 2016), cucumber mosaic cucumovirus resistance (Dogimont *et al.* 2000), aphids and whiteflies resistance (Boissot *et al.* 2010).

Phenotyping of low-heritability, polygenic quantitative traits unfortunately are typically difficult. Measuring tolerance to abiotic stresses are not simple because a relatively small shift on the stress level may lead to a dramatic change on the phenotype. Even more difficult is that, another stress (e.g. heat) can be confounded with the stress-of-interest (e.g. drought) if the experiment is not controlled properly. Utilization of computer-aided, precision phenotyping which are becoming more popular could be a good opportunity for obtaining more accurate phenotypic data. Some of the tools are not very expensive (for example, using scanner and/or computer imaging devices along with a relevant software for quantifying color components as RGB) and could be of practical use in a breeding program.

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In vitro shoots multiplication of Sapodilla (*Manilkara zapotta* Van Royen) with modified MS media

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Abstract

Sapodilla is a fruit exotic from a tropical country that has a sweet taste and good for healthy. Initiation of sapodilla'shoots by in vitro propagation has been widely reported but in vitro multiplication of sapodilla still limited report. The aim of this research was to in vitro multiplication of shoot of sapodilla with modified MS media. Explants of sapodilla were collecting from Serang area, Banten Provence and were induced of shoots with in vitro methods. Shoots grown from induction media were cut and planted at modified MS media contain BAP and Kinetin with J1, J2, J3 and J4 treatments. The results showed that with JI treatment the average of multiplication rate was 5.33 shoots/plant, following by J3 treatment with 4.60 shoots/plant, J2 treatment with 4.07 shoots/plant and J4 treatment with 1.8 shoots/plant. The rate of multiplication of shoots was decreased when the high concentration of BAP was added.

Keywords: Sapodilla, Manilkara zapotta Van Royen, in vitro multiplication

1. Introduction

Manilkara zapota or *Achras sapota* (Sapotaceae) commonly known as sapodilla is a fruit exotic plant from tropical countries, native to Yucatan and possibly other nearby parts of southern Mexico (Morton, 1987; Wasielewski and Campbell, 2001; Moo-Huchin *et al.* 2013; Peiris, 2014). Sapodilla has now spread to almost all tropical countries of the world (Jalawaadi *et al.* 2013). Based on Meghala *et al.*, In Asia, it was first introduced to the Philippines by the Spanish and later spreads to other Asian countries including Indonesia. Sapodilla is an important component of estate agriculture for sale in local markets in both Tropical Asia and America. Increasingly the sapodilla is grown on a large commercial scale in Central America, Brazil, India, Thailand the Philippines (Campbell and Noris, 2002), Sri Lanka, Pakistan, and Palestine, Malaysia, Mexico, Venezuela, Vietnam, Guatemala, and some other Central American countries (Peiris, 2014). In fact, India is one of the largest producers of Sapodilla.

In Indonesia, sapodillas are classed in two main groups: 1) Sawo maneela, normal size trees having narrow, pointed leaves; and 2) Sawo apel, low, shrub-like trees, with oblong leaves broadest above the middle (Morton, 1987). The data from BPS (2016) showed that production of sapodilla fruit in Indonesia 134.647 tons in 2015 was decreased if compared to 2014 (Figure 1). Sapodilla cultivated almost at all provinces but the central production of sapodilla in Indonesia dominated by 3 provinces, i.e.: West of Java, Lampung, and Central of Java provinces (Table 1). Most of the sapodilla plants in Indonesia are prevalent in home gardens and some area cultivated seriously for commercial.

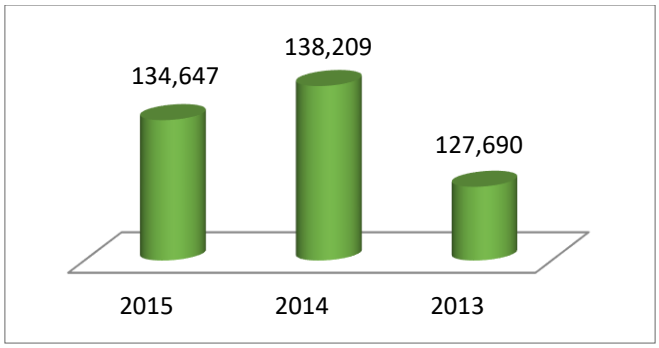

Figure 1. Production of sapodilla in Indonesia for 3 years (9).

Table 1. Production of sapodilla in Indonesia based on provinces

No	Provinces	Production of sapodilla (tons)		
		2015	2014	2013
1.	West of Java	19670	22308	16341
2.	Lampung	15657	15468	14012
3.	Central of Java	12470	13558	12792
4.	East of Java	12100	12400	9535
5.	West of Nusa Tenggara	11828	11335	9271
6.	West of Sumatera	10173	10584	9903

Source: BPS, 2016

Sapodilla is a long life evergreen tree that can reach 30-40 m in height (Rocas; Singh and Bothara, 2014). Fruit of sapodilla mainly used as the fresh fruit in most of Country but in Central America also used as processed fruit (Campbell and Noris, 2002). Sapodilla is a highly delicious fruit, sweet in taste, with a slight astringency. Its pulp is soft and crumbling with a sandy, granular texture and its aroma is very delicate, edible, and sweet to very sweet (19-24° Brix) with rich fine flavor (Peiris, 2014; Pino *et al.* 2003; Nagani *et al.* 2012). Fruit of sapodilla also one of the higher sugar fruits, but contains a healthy dose of iron, which keeps energy levels sustained and transports oxygen to the blood. Fruits were reported to contain polyphenolic compounds that showed antioxidant, antihyperglycemic and hypocholesterolemic activities (Fayek *et al.* 2013). Sapodilla contains magnesium, which keeps bones healthy, stabilizes blood pressure, and maintains nerves. Niacin, also found in sapodilla, reduces arthritic pain, promotes healthy circulation and assists with the body's natural energy production. As the source of chicle, the elastic gum which is made from the latex of the bark and which was the main ingredient of chewing-gum (Malo, 1967). High fiber content, sweetness, and high nutritional value have resulted in commercial production of sapodilla (Chaughule *et al.* 2011).

Sapodilla propagates naturally by seeds (Rocas). Propagation of sapodilla by seed gives low productivity and a lower fruit quality. Since there is great variation in the form, quality, and yield of fruits from seedling trees, vegetative propagation has long been considered desirable but has been hampered by the gummy latex (Morton, 1987). Commercially, sapodilla propagated by aerial shoots, stem cutting, or grafts (von Carlowitz 1991 in Rocas). In India, several methods are practiced: grafting, inarching, ground layering and air layering (Morton, 1987). The success rate of air layering is very low and even if successful, a layered branch takes few months to produce roots making this method time consuming and uneconomical (Purohit *et al.* 2007). In vitro propagation is one of the vegetative propagation methods, in sapodilla has been reported by many of researcher (Purohit and Singhvi, 2004; Dave and Purohit, 2004; Purohit *et al.* 2004; Purohit *et al.* 2007; Yuniasatuti *et al.* 2016; Wardani,

2016; Faqiha, 2016). Initiation of Sapodilla' shoots by in vitro propagation has been widely reported (Purohit and Singhvi, 1998; Purohit *et al*. 2004; Yuniastuti, 2016), but in vitro multiplication of sapodilla still limited report (17, 18, 20, 22, 23). The aim of this research was to in vitro multiplication of shoot of sapodilla with modified MS media.

2. Materials and Methods

Materials used for this research for explants were cutting off in vitro shoots of *Manilkara zapotta* collected from Serang area, Banten Provence 2-month-old. Media used for this research was modification Murashige and Skoog- MS (24) media supplemented with Benzyl Amino Purine (BAP) and Kinetin as growth regulator with J1 (4 mg/L BAP, 4 mg/L Kin), J2 (4 mg/L BAP), J3 (6 mg/L BAP) and J4 (8 mg/L BAP) treatments. For modification of MS media, 2x concentration of NH_4NO_3 from MS was used and added Calcium pantothenate and Biotin for each media.

Shoots from initiation media were cut approximately 3-5 cm then planted at each media treatments. Incubation all induction of multiplication shoots at 25-28^oC temperature and observes multiplication shoots every 2 weeks until 12 weeks.

The parameter for observations includes: percentage of shoots grown, the average of shoots multiplication rate, the average of leaves, the average of length of shoots, and the average of nodes.

3. Results and Discussions

We used cutting of shoot resulted from initiation for explants. Initiation for shoot used nodes for explants from sapodilla that planted at Serang area, Banten Provence. Shoots from in vitro initiation of nodes approximately 5-6 cm of height were cutting approximately 2-3 cm for induction of shoots multiplication (Figure 2). Then sub culture shoots were incubation at thermostatic room with 25-28^oC for grown and observed.

Figure 2. Explants used for shoots multiplication of sapodilla 2-month-old

The research showed that initiation of shoot for multiplication was beginning at 2 weeks after sub culturing in media treatments (Figure 3a) with 0.5 cm of height. Time for induction of new shoot more quickly compared with (17) reported till 3 weeks at standard media containing 2.0 mg/L BAP. Initiation of shoot multiplication was slowly until 2 weeks left to be observed. The percentage of shoots to grown after sub culture almost 80% more, except on J3 media.

Percentage of shoots grown on modification media showed that on J1 media shoots have capable to grown better than another media with 83.33%, while on J3 media had less response to grown with 62.50% (Table 2).

Table 2. Percentage and number of shoots grown

Media	Percentage of shoots grown (%)	Multiplication rate
J1	83.33	5.33 ± 3.54
J2	82.35	4.07 ± 2.30
J3	62.50	4.60 ± 2.97
J4	80.00	2.90 ±1.89

Figure 3. In vitro grown of shoots of sapodilla, after subculture 2 weeks (a), 6 weeks (b) 8 weeks (c) and 12 weeks (d)

The average of multiplication rate for sapodilla on J1 media was 5.33 ± 3.54. These media give the best multiplication rate compared to the other media. The combination of BAP and Kinetin were added on media, produce the maximal multiplication in this research. The rate of multiplication of shoots was decreased when the high concentration of BAP was added (Table 2). For this research maybe concentration of BAP was used too high. If compared with (17), concentration of BAP used was 2.0 mg/L BAP and obtained six shoots per node on Schenk and Hildebrandt (SH) basal media but if on Woody Plant Media (WPM) basal media concentration of BAP 2.0 mg/L just induced 1,67 shoots per node (23). We assume that basal media used maybe effecting the induced of shoots multiplication on sapodilla. Improvement in growth and multiplication of shoot culture was reported by (18) used controlled carbon dioxide environment with results showed that the best response was obtained at 10.0 g m-3 CO2 where maximum number of shoots (average 8.66) with highest shoot length, leaf number and leaf area

was obtained at 42 day of culture. Incorporation of GA3 at 1.0 mg/L with 2.0 mg/L BAP not only induced shoot elongation but also enhanced the rate of multiplication of sapodilla (ca. six shoots per node) (17) or with Putrescine (a polyamines) added 0.1 mM and BA (8.87 µM) a 3-fold rate of shoot multiplication was achieved where an average of 5.17 shoots per nodes were obtained (20).

Table 3. Average of number of leaves, nodes and height of shoots

Media	Average number of leaves	Average number of nodes	Average of height (cm)
J1	7.84 ± 5.54	6.89 ± 3.59	1.70 ± 0.77
J2	6.07 ± 2.36	5.22 ± 2.40	1.27 ± 0.72
J3	6.34 ± 2.46	5.26 ± 2.32	1.07 ± 0.62
J4	8.58 ± 3.11	7.29 ± 3.25	1.63 ± 0.72

The average number of leaves showed that J4 media give the best media for induced number of leaves at sapodilla shoots (Table 3) with 8.58 ± 3.11. Results showed that more BAP added to media capable to induce leaves, otherwise the less concentration of BAP added number of leaves were reduced (J2 and J3 media). At J1 media, if media added with BAP combination with Kinetin, even BAP added with concentration 4 mg/L, the average number of leaves almost same with J4 media because Kinetin was added at J1 media that can improve number of leaves. The results showed that an average number of nodes same with number of leaves with J4 media was the best media and an average number of node was 7.29 ± 3.25.

Figure 2. Shoots multiplication of sapodilla 12-weeks-old

The height of shoots was showed that J1 media has the best media of inducing shoots height with average 1.70 ± 0.77 followed by J4 media with 1.63 ± 0.72 (Table 3). As we seen that node from J1 media was 6.89 ± 3.59 and the height of shoots just 1.70 ± 0.77, so the length between nodes was short. Maybe added GA3 with 1.0 mg/L at media as (17) reported could add the length between nodes.

For this research we assume that media J1 has the same effect with media J4 for percentage of shoots grown, the average number of leaves, nodes and height of shoots but no with multiplication rate. If we used 4 mg/L BAP at media, so we must add Kinetin to optimal grown but if we are not used Kinetin, we can use BAP with 8 mg/L at media MS modification. The used MS modification with 2x concentration of NH_4NO_3 showed that shoots of sapodilla can multiply. Modification of MS media also had been reported to multiply teak shoots (25) by 50% reduction in NH4NO3 concentration.

4. Conclusion

The best media to multiply shoots of sapodilla was J1 media. The average of multiplication rate for sapodilla was 5.33 ± 3.54. The rate of multiplication of shoots was decreased when the high concentration of BAP was added. The used MS modification with 2x concentration of NH_4NO_3 showed that shoots of sapodilla can multiply.

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Confirmation Number of Chromosome Diploid, Autotetraploid and Triploid Hybrid 'Rejang' Banana Using Digested Anther

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Abstract

The Results from plant breeding activities that have been carried out at Research Center for Biology LIPI has obtained tetraploid Rejang banana (results from doubling chromosomes of diploid Rejang using oryzalin compound) and triploid hybrid Rejang banana (result of crossbreeding mixoploid Rejang with diploid Rejang banana). Analysis of ploidy level of banana previously has done using flow cytometry. The ploidy level in banana from plant breeding activities also needs confirmed by counting the number of chromosome. Confirmation number of chromosome is done using digested anthers with chromosomes stained using DAPI (4'6-diamidino-2-phenylindole). Procedures involved; young flower buds were harvested, anther dissected from flower (sizes from 0.5 to 1 cm), fixation with acetic acid-alcohol, enzymatic digestion of cell walls, chromosomes stained using DAPI, and observations under fluorescence microscopy. The results of chromosomes counting from digested anther confirmed that diploid Rejang chromosome number 2n = 2x = 22, triploid hybrid Rejang 2n = 3x = 33, and tetraploid Rejang 2n = 4x = 44. This procedures ease in sample preparation, saving time to determination the correct stages of cell division, plant cells not overlap and the chromosome spread, so that easily to counting.

Key words: chromosome number, ploidy, banana/ musa, digested anther, DAPI

1. Introduction

Banana and plantain (*Musa* sp.) is one of the fruits are widely consumed in Indonesia, where the genetic diversity of Musa are commonly found both cultivated and wild. Genetic modification and breeding to create new varieties with desired characteristic is therefore of interest to research. Crop improvements through ploidy manipulation in banana cultivar are widely used to increase biomass/ productivities or to provide tetraploid parent for crosses with diploid parent to create triploid hybrid. Polyploidization is an important process and has been utilized to breed new cultivars with horticultural characteristic such as disease-resistance, large fruit size, sturdiness and seedless fruits (Kanchanapoom and Koarapatchaikul, 2012).

'Rejang' (*Musa acuminata* Colla (AA genomes)) is dessert banana, one of Indonesian local cultivar that can found in Sumatra and Java Island. The Results from plant breeding activities that have been carried out at Research Center for Biology LIPI has obtained tetraploid Rejang (results from doubling chromosomes of diploid Rejang using oryzalin compound) and triploid hybrid Rejang (result from crossbreeding mixoploid Rejang with diploid Rejang banana)

(Poerba *et al*. 2016).

Previously, the ploidy level of Rejang (diploid, triploid and tetraloid) was analyzed by flow cytometry. In addition, ploidy level need confirmed by counting the number of chromosome. Flow cytometry used in order to detect changes in the genome size or ploidy, since there are advantages of speed, efficiency, accuracy, and technical simplicity for determining ploidy levels by assessing the DNA content (Kanchanapoom and Koarapatchaikul, 2012; Kron *et al*. 2007).

In general, ploidy level detection by conventional methods e.g., chromosome counting, stomata size measurement requiring a microscope only. The main problems associated with preparation of good chromosome spreads, and the small size and poor staining ability of prophase on *Musa* chromosomes. Musa chromosomes are only 1—2 µm in length at mitotic metaphase. Chromosome counting using root tip squash was complicated by time consuming efforts of finding the correct stages of cell division (Adeleke *et al*. 2002). Preparation method improvement has been established in meiotic studies using anther and various stains for musa chromosome (Adeleke *et al.* 2002; Dolezel *et al*. 1998). This paper aimed to confirmed the number of chromosome on Rejang Banana with difference ploidy level result from plant breeding, using digested anthers and chromosomes stained using DAPI (4',6-diamidino-2-phenylindole) staining.

2. Materials and methods

Plant materials. The genetic material used in this study included diploid Rejang (AA), hybrid triploid Rejang (AAA), and autotetraploid Rejang (AAAA) maintained in germplasm garden collection Research Center for Biology - LIPI in Cibinong Science Center, Bogor. Genetic materials were selected to represent difference ploidy level within Rejang accessions. Previously, ploidy level observation using flow cytometry, with protocol analysis in *Musa* sp. (Dolezel and Barton, 2005).

Number of Chromosome identification. The protocol was conducted based on Adeleke *et al.* (2002) with some modification developed in Plant Genetics and Breeding Laboratory, Research Center for Biology LIPI. Selected of anther were harvested from young male buds on sunny days between 08.00—10.00. Anthers were dissected from floral buds and fixation in 3 ethanol : 1 acetic acid solution for 30—60 min, then select anther with length range from 0.5—1.0 cm washed 3 times in distilled water and placed in 2 ml tube, then added ± 200 µl enzyme mixture (approximately 2—2.5 x anther volume). Enzyme mixture (1% *cellulase* RS, 1% *pectyolase* dan 1% *cytoglikase*) prepared in citrate buffer (10mM Na-Citrate buffer, pH 4.5). Cell wall digestion by incubated in enzyme mixture at 37°C for 2 h. The digested anther were washed 3x using cold distilled water (ddH_2O) and add 0.5 – 1 ml fixative solution (methanol: acetic acid = 3:1).

Slide preparation—flame dry method. Transfer 1—2 digested anther with approximately 30 – 50 µl fixative solution to a clean microscope slide. Use very fine needle forceps to macerate the anther thoroughly. While macerating/ chopping the anther, also gently spread the suspension by using the very fine needle forceps, add a drop of fixative solution and quickly crossing the flame of the burner. Then stained with DAPI (4'6-diamidino-2-phenylindole) (1 DAPI: 20 *vectashield*) ± 15 µl to slide, and closed with glass cover. Slides with well spread chromosome were photographed in fluorescence microscope (Olympus BX53) using 100 x 1.35 oil immersions.

3. Result and Discussion

This study aimed to confirmation ploidy level of Rejang banana using chromosome counting that previously analysis using flow cytometric. Accurate information of ploidy and genome classification was important for banana/ musa breeding programs, with *Musa* spp (bananas and plantains) constitute a hybrid-polyploid complex and are classified according to different genome compositions such as AA, BB, AB, AAA, AAB, ABB, AAAA, ABBB, AAAB, AABB and BBBB. Knowledge of ploidy level and exact genome compositions of the parental material is essential for Musa breeding (Pillay *et al*. 2006).

In this study, ploidy level was first estimated using flow cytometry, because is convenient and rapid method for the detection of polyploidy and aneuploidy in musa species (Roux *et al*. 2003). Analysis of ploidy level using flow cytometry on banana population especially for Rejang accessions from garden collections of musa germplasm at Research Center for Biology – LIPI Cibinong, result about 18 plant clumps are diploid, 27 plant clumps are tetraploid, and 33 plant clumps are triploid (Poerba *et al*. 2016).

Flow cytometry histogram representative from selected Rejang with different ploidy level displayed in Fig. 1 (a-c). The flow cytometry measurement revealed three types of histograms, in diploid accessions peak channel was approximately on channel 200 (Fig. 1a, peak channel mean-x=214.74, CV=7.67%), triploid showed a peak channel approximately on 300 (Fig. 1b, peak channel mean-x=214.74, CV=8.55%) and tetraploid showed a peak channel approximately on 400 (Fig. 1c, peak channel mean-x=373.84, CV=7.30%).

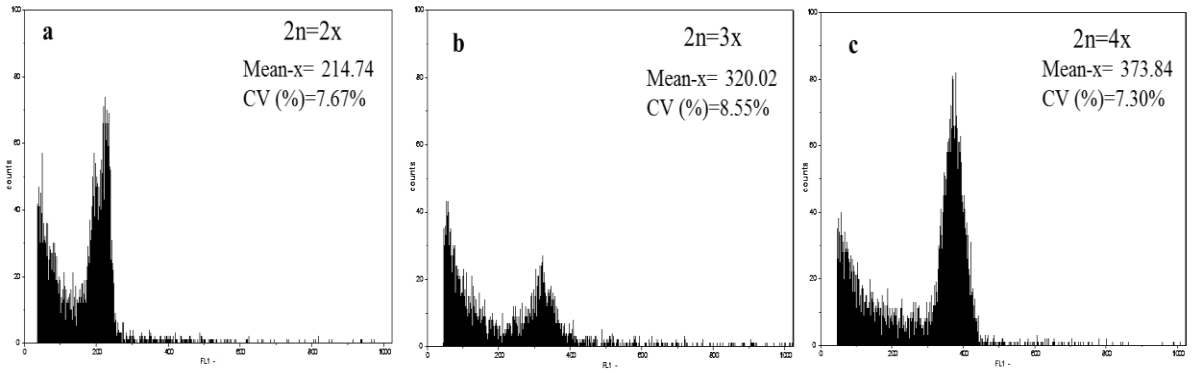

Figure 1. Representative histogram from ploidy flow cytometry analysis of Rejang accession. The figure shows histogram of relative nuclear DNA content (a) diploid profiles (2n=2x); (b) triploid profile (2n=3x); (c) tetraploid profile (2n=4x)

Flow cytometric analysis also known as rapid method on large population of nuclei (5 - 10.000) and even sub-population differing in ploidy level (mixoploid/ aneuploidy) (Asif *et al.* 2001). Although flow cytometry proved to be efficient for ploidy determination, however information regarding the chromosome study on difference accessions is still required for breeding program, especially for basic information on cytogenetics, e.g. karyotype study, structural chromosome change, or study on mechanism crossing – over during cell division (Schwarzacher, 2016) or study in plant genome structure.

Cytological analyses using anthers/ male inflorescence made it possible to observing all stage of mitotic or meiotic phases on pollen mother cells in musa species including early prophase stage that shows pachytene chromosome (Adeleke *et al*. 2002). Our observation on companion cell somatic from digested anther shows that morphology of chromosome some stage of mitotic phase are clearly shown (Fig. 2), with the chromosomes appear sharply defined, not overlap and stained clearly. Chromosomes usually easy to count on late prophase (sometime

also called prometaphase) because in this phase the chromosomes finish condensing, so they are very compact, and chromosome appear more sharply.

DAPI showed effective for staining mitotic chromosome that appeared clearly under fluorescence microscope. DAPI (4'6-diamidino-2-phenylindole) is one of various chromosomes stain that used extensively in fluorescence microscopy with its emission maximum is at 461 nm (blue emission), binds strongly to A-T rich region in DNA, can pass through an intact cell membrane, making easy to detect the chromosome under fluorescence microscope (Beccia *et al*. 2012). DAPI staining usually used to study fluorescence in situ hybridisation (FISH) has considerably contributed to a better understanding of plant genome structure and evolution (Valarik *et al*. 2004). Giemsa, Leisman's stain, silver staining are other staining were also effective for meiotic or mitotic chromosome stain of Musa especially for the well – condensed metaphase and anaphase chromosome including for prophase chromosome (Adeleke *et al.* 2002).

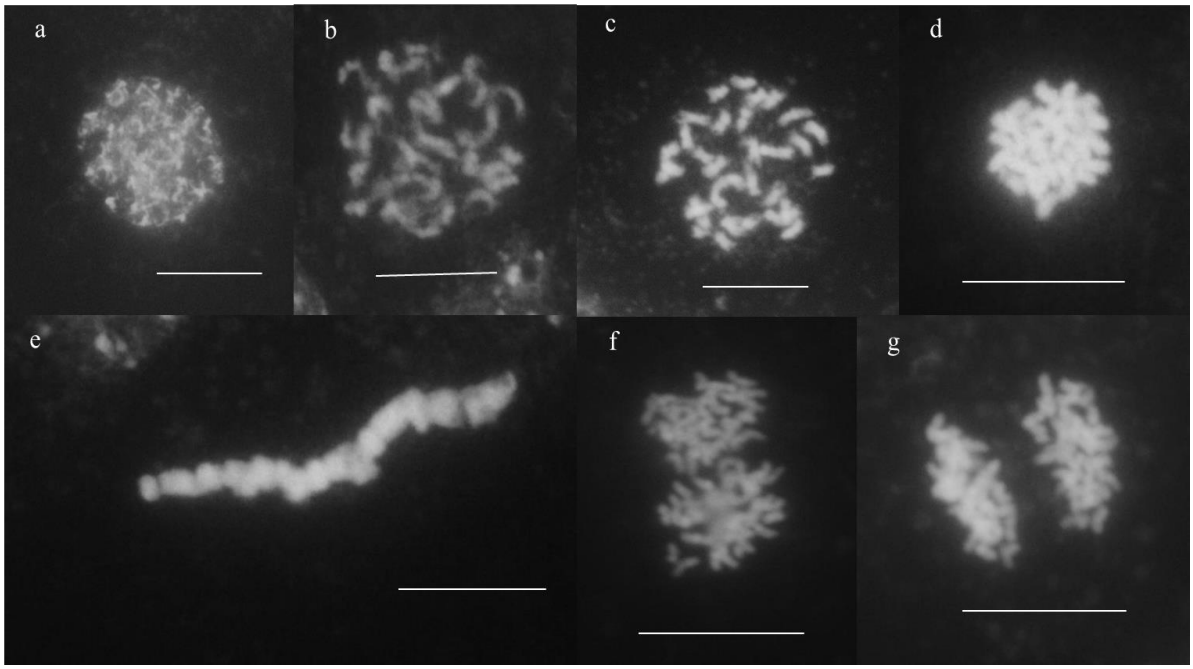

Figure 2. Appearance some mitotic phases of cell division from digested anther under fluorescence microscope, a-d) The chromosome (paired chromatids) are becoming visible, chromatin condenses into chromosome, chromosomes are shortened and thickened (prophase); e) chromosome line up on equator of cell (metaphase); f-g) two new cell in interphase result. Bar = 10 µm.

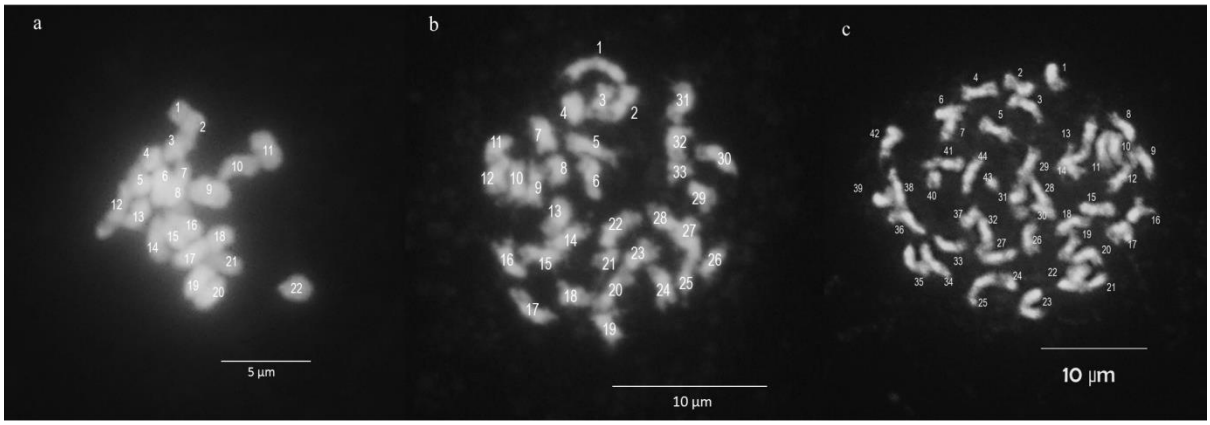

Figure 3. Chromosome number observation of Rejang accession; (a) diploid cell with 2n = 2x = 22; (b) triploid cell with 2n = 3x = 33; (c) tetraploid cell with 2n = 4x = 44

In this study, number of chromosome counting from companion cells anther that its easily found in microscope slide than pollen mother cell. A simple purpose study for example to determination number of chromosome or karyotype study, mitotic chromosome on companion cells that found spread well (with correct stage of cell division) on slide can be used to analysis. The results of chromosomes counting confirmed result from flow cytometric analysis that diploid Rejang chromosome number 2n = 2x = 22, triploid hybrid Rejang 2n = 3x = 33, and tetraploid Rejang 2n = 4x = 44 (Fig. 3). Using this method for determine chromosome number was saving time to make good slides compared using root tip squash that time consuming to finding the correct stages of cell division, and need required special skills to make good slides.

4. Conclusion

The results of chromosomes counting from digested anther confirmed that diploid Rejang chromosome number 2n = 2x = 22, triploid hybrid Rejang 2n = 3x = 33, and tetraploid Rejang 2n = 4x = 44. The procedures using male inflorescence to chromosomes analysis are ease in sample preparation, saving time to determination the correct stages of cell division, plant cells not overlap and the chromosome spread, so that easily to counting.

5. Acknowledgment

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Disease Incidence and Molecular Analysis of Banana Bunchy Top Virus in Bogor, West Java

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Abstract

Banana is a horticulture crops with economical value, cultivated in many types of tropical agricultural system. Banana bunchy top disease caused by *Banana bunchy top virus* (BBTV) infection is considered as the most important virus disease affecting yield losses of banana plantation in Asia, Africa, and South Pacific. Previous study reported that BBTV diversity hotspots located and originated in South East Asia, including Indonesia. However, the incidence and molecular characters of BBTV in Indonesia is still slightly understood. The objective of this research was to characterize symptom variations, disease incidence, and study molecular characters of BBTV from banana plantations in Bogor. Disease incidence of BBTV was measured based on field symptoms. Infection of BBTV were confirmed using immunocapture-polymearse chain reaction (IC-PCR). Further molecular characterization of BBTV was performed based on analysis of viral nucleotide sequences. The study showed that incidence of BBTV in Bogor Districts ranged from 3.3% to 28.4%. The most common symptoms observed in the field involved vein clearing, upturned leaf, chlorotic, and ragged margins with reduction in petiole length, distance, and lamina width. Molecular characterization of DNA-R and DNA-S of the samples confirmed that BBTV isolates from Bogor belongs to Asian group, and distinctly separated from those of South Pacific group. Variety of BBTV among isolates from Bogor is not very high, with sequence homology ranged from 97.2% to 99.3% and 96.6% to 100% for DNA-S and DNA-R, respectively.

1. Introduction

Banana is one of the world's most important horitculture crop, cultivated in many types of tropical agricultural system. It is the fifth most traded horticultural product in international market (Aurore *et al.* 2009). Most of the currently cultivated bananas are diploid or triploid that belong to Eumusa section, hybrids from *Musa acuminata* (A-genome) or from hybridization with *Musa balbisiana* (B-genome). The large-scale vegetative propagation of small number of genotypes, which was derived from limited ancient sexual recombination, caused banana clones are particularly susceptible to disease, pest and ecological changes (Perrier *et al.* 2011).

Banana bunchy top disease (BBTD) caused by infection of *Banana bunchy top virus* (BBTV) is considered as the most important virus disease affecting banana plantation in Asia, Africa, and South Pacific. Infection of BBTV can cause up to 90% yield lost (Dale, 1987). The disease have caused a big destruction on banana plantation in Australia, and currently become a big problem in Africa. The incidence of BBTD in Indonesia have been reported in Sumatra, Bali, and Special Territory of Yogyakarta (Furuya *et al.* 2004; Pinili *et al.* 2011; Chiaki *et al.* 2015).

Infection of BBTV spread through infected banana sucker and other plant tissue that used for banana propagation, only transmitted persistently by its specific vector, banana aphid,

Pentalonia nigronervosa (Magie, 1927). Dark green streaks underneath the lamina and petiole was reported as the early symptoms, followed by production of stunt and malformed leaves with pale chlorotic margins, eventually resulting 'bunchy' appearance. Early infection of BBTV in banana caused no fruit production, while late infection produces stunted and unmarketable fruits (Thomas, 2008). Incubation period of BBTV ranged between 25 days and 85 days after inoculation (DAI), with significant suppression of plant growth in 50 DAI (Hooks *et al.* 2008).

BBTV is now recognized as a member of Genus *Babuvirus* in Family Nanoviridae, containing at least six genome components, each approximately 1000 bp size. The six genome components reffered to DNA-R, DNA-U3, DNA-S, DNA-M, DNA-C, and DNA-N, encoding different types of protein (King *et al.* 2011). All six components shared two common region, major common region (CR-M) and stem-loop common region (CR-SL) that associated with the conserved stem-loop structure (Burns *et al.* 1995).

BBTV isolates in the world fall into two geographic phylogenetic groups, i.e. the South Pacific group (SPG) and Asian group (AG) based on genome components (Karan *et al.* 1994). The isolates collected from South East Asia form a high degree of geographic clustering in AG, while almost all sequence sampled outside the region falling into the SPG. Previous study on distribution of BBTV have indicated that global BBTV diversity hotspots located and originated in South East Asia, including Indonesia (Stainton *et al.* 2015). However, the epidemiology and molecular characters of BBTV in Indonesia is still slightly understood. This research aimed to analyze the incidence and molecular characters of BBTV in Bogor, Indonesia.

2. Methods

Field Survey and Sample Collection. Samples were collected during survey from several banana plantations in Bogor, West Java. Survey areas covered several sub-districts i.e., Cimayang and Cikoneng (Sub-district Pamijahan), Barengkong (Sub-district Leuwiliang), Kalong (Sub-district Leuwisadeng), Bontar (Sub-district Cigudeg), Pasar Ciampea (Sub-district Ciampea), Kota Sawah (Sub-district Rumpin), Cagak (Sub-district Rancabunggur), Parung (Sub-district Parung), and Cikabayan (Sub-district Dramaga). Infection of BBTV was observed using purposive sampling method based on BBTV common symptoms i.e., stunting, bunched up leaves, and streaking between leave margins and midrib. Banana leaves were put into a labeled plastic wrapper and kept inside the ice box before transferred into laboratory. Fresh tissue was directly subjected for virus detection and the remainings were stored at -80 ^{o}C as isolate collection in the laboratory. Disease incidence (DI) of BBTV in each plantation was calculated using the following formula:

$$\mathrm{DI} = \frac{\sum \text{symptomatic plant}}{\sum \text{total banana population}} \times 100\%$$

Based on the severity of the symptoms, the level of infection was categorized into three groups, i.e mild, intermediate, and severe infection. This grouping system was then used to classify banana plant samples during the survey.

Immunocapture-polymerase chain reaction (IC-PCR). IC-PCR was conducted to confirm the infection of BBTV from leaf samples. Thin-wall polypropylene PCR tubes was coated with 100 µL BBTV antibody (Agdia Inc.) in coating buffer pH 9.6 (1:200 dilution) and incubated overnight at 4 ^{o}C. After washing the tubes, 100 µL plant sap was added into individual PCR tubes (each 100 µL), and incubated 3 h at room temperature (± 37 ^{o}C). Plant sap was then

discarded flow-through by knocking on the paper tissue pad then washed using 100 µL PBST 1x containing 1% Triton-X. After this step total viral DNA was trapped on the tubes. Resuspension of total viral DNA was done by adding 25 µL of dH2O into PCR tube and the DNA can be used as a DNA template for further PCR amplification using two specific primer pairs as described on Table 1.

Table 1. Specific primers for amplification of BBTV

Target DNA	Primer	Sequence	Amplicon size	Reference
DNA-S	CP1/F	5'-CCCGGGAGAATACTTCACTGGGCTATGATT-3'	1083 bp	Mansoor *et al*. 2005[19]
	CP1/R	5'-CCCGGGCTTCACCTTGCACACCAACAGCAT-3'		
DNA-R	mRep/F	5'-GCGTGAAACGCACAAAAGGCC-3'	240 bp	Amin *et al*. 2008[20]
	mRep/R	5'-GCATACGTTGTCAAACCTTCTCC TC-3'		

Amplification of DNA was conducted based on method described by Kumar *et al*. (2011). The DNA was amplified in GeneAmp PCR system 9700 machine with 5 min at 94 ºC for pre-heating, followed by 35 cycles of denaturation (30s at 94 ºC), annealing (45s at 55 ºC), and extension (30s at 72 ºC), with final extension of 7 min at 72 ºC. Amplicons was then visualized on 1% agarose gel using electrophoresis in TBE 0.5x buffer. Following the electrophoresis process, agarose gel then soaked on to 0.1% EtBr for 15 min, washed with H_2O for 5 min, and visualized under UV transilluminator.

Nucleotides Sequence and Phylogenetic Analysis. PCR products then sequenced at 1st BASE Laboratories (Malaysia). Sequence contigs were assembled using CLC sequence viewer 7.5, then aligned with sequence isolates from GenBank using Bioedit 7.2.5 to analyze the sequence homologies. GenBank sequences with accession number of KM607442, KM607493, HQ378191, KM607446, EU589459, KM607447, KM607570, and KM607465 was used for DNA-S analysis; whereas KM607588, KM607637, HQ378190, KM607593, EU140342, KM607594, KM607710, and KM607608 was used for DNA-R analysis. The sequence of *Abaca bunchy top virus* (ABTV), i.e. EF546810 and EF546813 for DNA-S and DNA-R, respectively was used as the outgroup comparison. Phylogenetic three was constructed using program ClustalX, Bio Edit 7.2.5, and MEGA 6.12.

3. Result and Discussion

Disease Incidence (DI). Banana plantations in Bogor were cultivated as a main crop in monoculture or intercrop culture system with other horticultural crops such as papaya, cassava, or in some cases durian and estate crops. The highest incidence of BBTD was observed in Kalong, Leuwisadeng (LS) with DI value 28.4%, while the lower DI were observed in three other plantations, i.e. Cikoneng (PM), Bontar (CG), and Cagak (RB) with DI value of 3.3, 3.4, and 3.5%, repectively (Fig 1).

A study of BBTV epidemiology in Burundi, Africa indicated that incidence of BBTD significantly varied according to location, banana cultivar and planting material (Niyongere *et al.* 2012). However, it is suggested that the higher incidence of BBTD can be attributed to the presence of large number of winged aphid (*P. nigronervosa*). Although another research have proved that inter-continental transfers of epidemiologically important BBTV genotypes have occurred through human-mediated infected plant transfers (Stainton *et al.* 2015), it is believed that banana aphid played a big role of transferring BBTV from infected plant to virus-free plant within the same cultivation area.

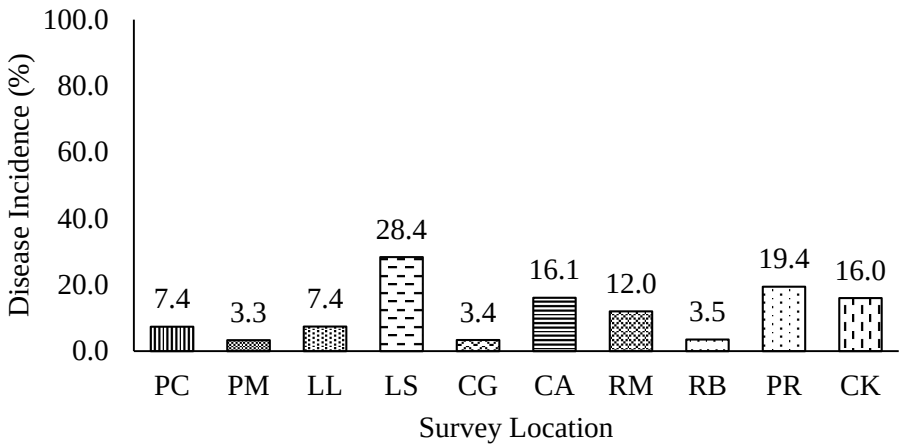

Figure 1. BBTV incidence in banana plantation in Cimayang (PC) and Cikoneng (PM), Barengkong (LL), Kalong (LS), Bontar (CG), Pasar Ciampea (CA), Kota Sawah (RM), Cagak (RB), Parung (PR), and Cikabayan (CK).

It is necessary to understand the distribution of BBTV infected plant on banana cultivation area for eradication and integrated management approach. It was suggested that safe distance for plot establishment is 30 m from existing disease field to prevent the early establishment of the virus and possible inoculum build-up (Niyongere *et al.* 2012). This is very important because disease incidence within banana plantation mostly influenced by the presence of aphid and distance of BBTV inoculum sources.

Symptoms of BBTV. Clear symptom of BBTV can be observed on the lamina of the leaves. The initial symptoms were characterized by the appearance of dark green streak and dots on petiole and lower part of lamina, also slightly chlorotic margins along the new developing leaves. However, the dark green streak can be absent from some cultivars and severe symptoms usually developed since the first leaf of plants derived from infected planting materials (Thomas, 2008). The general symptoms that observed in all banana plantation in Bogor involved upturned leaves, chlorotic and ragged margins, with leaves failed to emerge.

Based on type of the symptoms, infection of BBTV in Bogor can be differentiated into three category, mild, intermediate, and severe infection (Table 2). Not all of the symptoms type could be observed in every banana cultivation area in Bogor, but the highest diversity of symptoms was found in Kalong (LS). Based on dominant symptom type, infection of BBTV in Bogor was considered intermediate and severe. BBTV is restricted to phloem tissue, shows hypertrophy and hyperplasia and a reduction the development of the fibrous sclerenchyma sheaths surrounding the vascular bundles (Thomas, 2008). It was found that level of chlorophylls a and b and total chlorophyll is significantly lower in BBTV infected plants, resulting the slightly pale appearance and chlorotic margins (Hooks *et al.* 2008).

Table 2. Description of BBTV symptom severity

Symptoms Severity	Symptom Description
Mild infection	Limited vein clearing and dark green streaks on the lower part of lamina and on petiole. No significant reduction of lamina width.
Intermediate	Vein clearing, upturned leaf, chlorotic, and ragged margins. Significant reduction in petiole length, distance, and lamina width.
Severe	Brittle lamina with upturned, chlorotic, and ragged margins, sometimes with necrotic symptom. Leaves failed to emerged, giving a clear bunched appearance.

Table 3. Symptom severity based on banana cultivar

Cultivar	Symptom Severity			Confirmation by PCR
	mild	Intermediate	severe	
Tanduk	-	√	√	+
Ambon	-	√	√	+
Nangka	-	√	-	+
Kepok	√	√	√	+

The symptoms of BBTV also varies among banana cultivars (Table 3). However, all cultivars was found showing intermediate symptom. Mild infection can only be found in 'Kepok', while severe infection can be found in 'Tanduk', 'Ambon', and 'Kepok'. Previous research in Indonesia reported that 17 out of 38 banana cultivars that grown in banana germplasm field in Yogyakarta were infected by BBTV, with 5 cultivars ('Kepok Gabu', 'Raja Entos', 'Raja Trunpong', 'Rejang' and 'Tanduk Hijau') among them were positively infected without virus symptoms (Furuya *et al.* 2004). Although there were substantial effects on morphological and growth parameters of plant infected by BBTV, the symptoms are not unambiguous to naked eye until several weeks after inoculation. Incubation period of BBTV found varies in different places and under different cultivation condition, mostly 50 DAI (Hooks *et al.* 2008). There are no comprehensive report confirming any banana cultivars that completely resistant to BBTV infection. However, previous study have provided evidence that banana cultivars showed different response against infection of BBTV (Hooks et al. 2008; Niyongere *et al.* 2011).

IC-PCR of DNA-S and DNA-R. Twelve samples (PC1, PC2, PM2, LL, LS1, LS2, CG, CA, RM, RC1, PR1, and CK) from ten different banana plantations in Bogor were used for molecular characterization of BBTV. Fragment of BBTV DNA-R was successfully amplified under IC-PCR process using mRepF/mRepR primers in all samples with size of amplicons ± 240 bp. However, CP1F/CP1R primers targeting DNA-S (amplicons size ± 1083 bp) were able to amplify only nine out of twelve samples (Figure 2). Chiaki *et al*. (2015) suggested that inability to obtain sufficient BBTV DNA template is influenced among others by extraction process and condition of leaves samples. Similarly, Stainton *et al*. (2015) could only recover 94 complete BBTV genomes out of 171 infected banana plants from fourteen countries. The intensity of bands for DNA-S on the agarose gel was rather consistent for all nine amplified samples, while those for DNA-R was varied among all twelve samples, i.e. very strong (CA,

CG, PC1, PC2, PM2, and RM), strong (LL, LS1, and LS2), intermediate (CK), and weak (PR1 and RB1). It is possibly indicated the different titter of virus in leaf tissue.

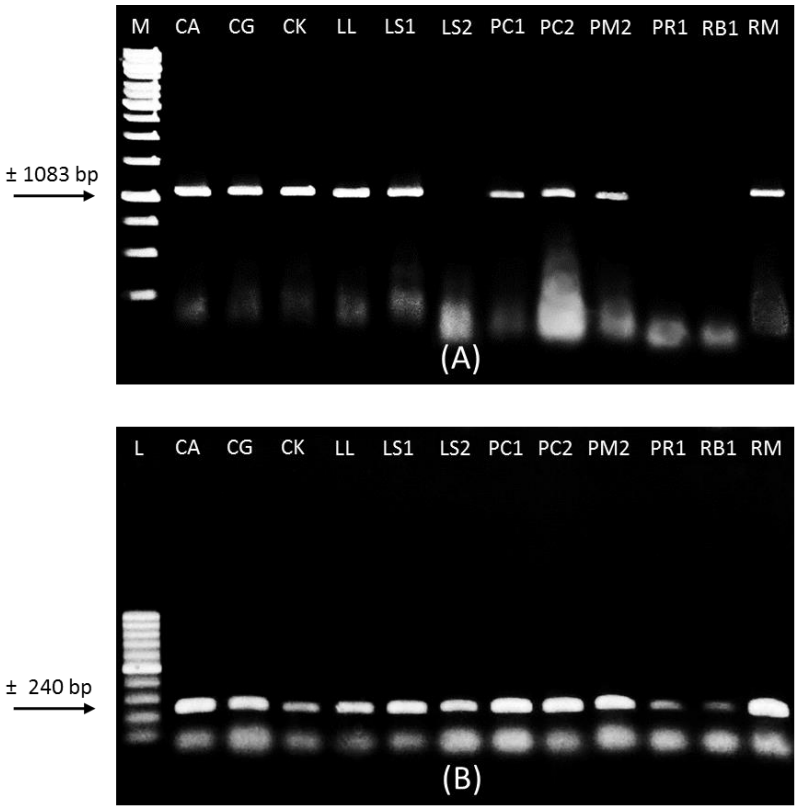

Figure 2. Visualization of PCR amplicons of BBTV Bogor isolates using CP1F/CP1R primers for DNA-S (A) and mRepF/mRepR primers for DNA-R (B). M, 1kb DNA marker and L,100bp DNA marker

Both molecular and serological detection method are important for rapid detection of BBTD incidence. Based on our result, it is suggested that using primer mRepF/mRepR on IC-PCR method is favorable for rapid detection of BBTV infection.

Nucleotides and Phylogenetic Analysis of DNA-S and DNA-R. All successfully amplified DNA fragment (six samples DNA-S and twelve samples DNA-R) were sent for sequencing, then compared with other sequence from GeneBank. All isolates were shown to have high homologies within the DNA-S and DNA-R (97.2% to 99.3% and 96.6% to 100%, respectively). Analysis of their identity by comparing it to other sequence from GenBank, BBTV Bogor isolates showed higher homology to isolates of AG than those of SPG (Table 4). Further analysis about their relationship showed that all BBTV isolates from Bogor belong to similar cluster and having closest relationship to isolate from Indonesia, Philippines, and Taiwan.

Table 4. Nucleotide sequence homology (%) of BBTV isolates from Bogor with other geographical isolates reported earlier in GenBank

Sequence comparison	BBTV genome component	
	DNA-S	DNA-R
Among Bogor isolates	97.2 – 99.3	96.6 – 100
Between Bogor isolates and the Asian group	98.0 – 99.3	94.2 – 99.5
Between Bogor isolates and the South Pacific group	82.4 – 84.2	83.8 – 88.4
Between Bogor isolates and *Abaca bunchy top virus* sequence	60.2 – 60.9	72.7 – 75.2

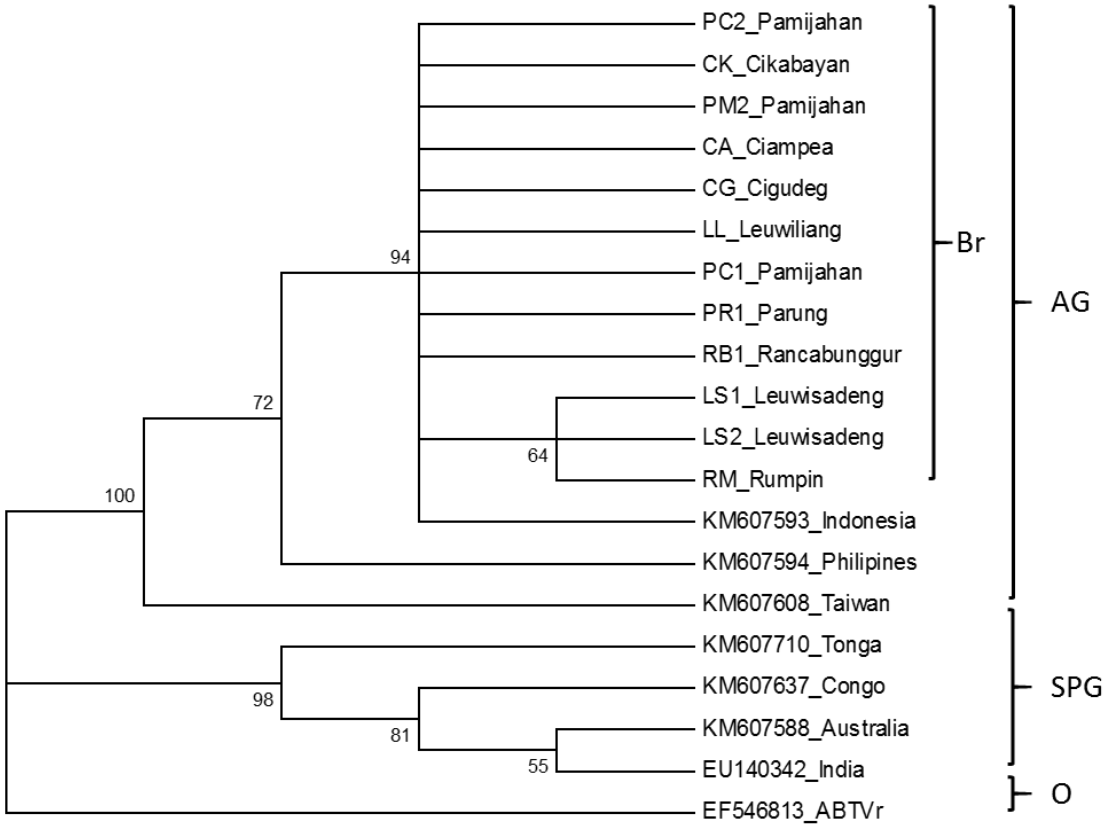

Figure 3. Phylogenetic analysis of BBTV isolates from Bogor (Br) based on nucleotide sequence of DNA-R. AG, Asian group of BBTV; SPG, South Pacific group of BBTV; O, ABTV as outgroup.

South East Asia was suggested as the BBTV diversity hotspots in the world, while the global distribution of BBTV was influenced by the human-mediated infected planting material transfers. Genetic diverity of all BBTV genome components, except DNA-S, within AG isolates was reported higher than SPG isolates (Stainton *et al.* 2015). Based on the phylogenetic analysis of DNA-R, samples from Kalong (LS1 and LS2) and Kota sawah (RM) form a stronger cluster inside the cluster of Indonesia and other samples from Bogor (Figure 3). Phylogenetic tree of DNA-S showed that BBTV Bogor isolates form a cluster with isolates from Indonesia, Philipines, and Taiwan (AG) and separatd from SPG isolates. This result suggested that BBTV Bogor isolates belong to AG.

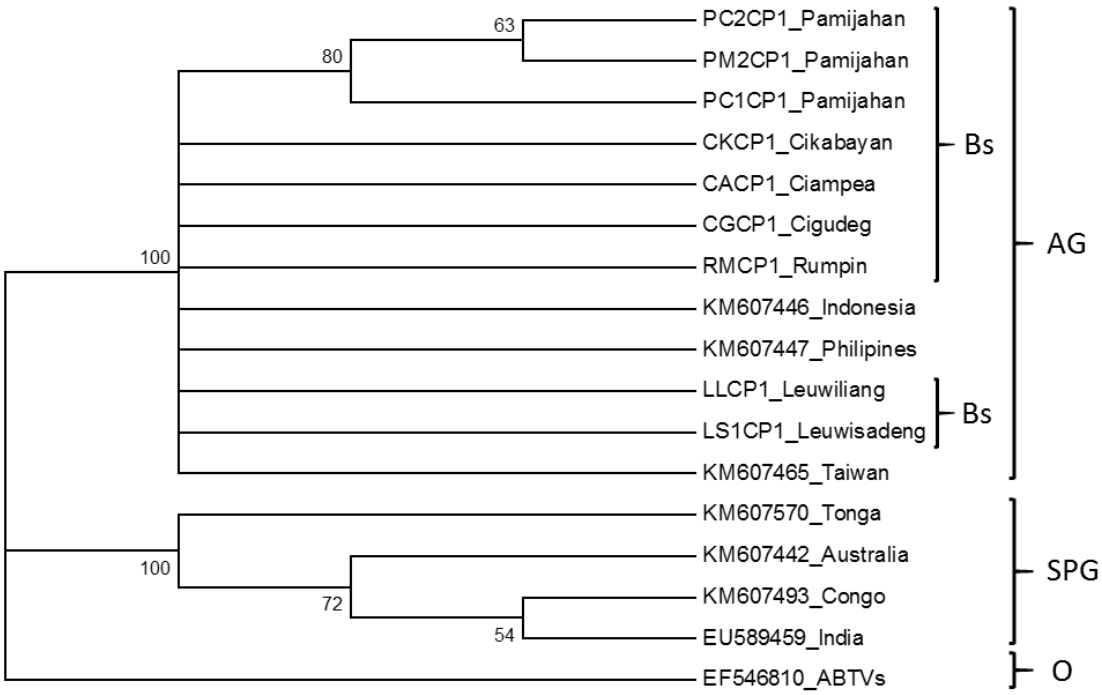

Figure 4. Phylogenetic analysis of BBTV isolates from Bogor (Bs) based on nucleotide sequence of DNA-S. AG, Asian group of BBTV; SPG, South Pacific group of BBTV; O, ABTV as outgroup.

4. Conclusion

Incidence of BBTD in Bogor is considered low based on symptom observation. However, most of infected plants showed intermediate to severe disease symptom involving upturned leaf, chlorotic, and ragged margins, also significant reduction in petiole length and lamina width. The phylogenetic analysis showed that BBTV isolates from Bogor has high homologies with other isolates from Indonesia, Philippines and Taiwan, confirming that it is belong to BBTV Asian group. Most importantly we found that mRepF/mRepR primers for DNA-R is more sensitive and suitable for rapid detection of BBTV using IC-PCR method.

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The Potential of Liquid Smoke Coconut Shell in Extending The Shelf Life of Tropical Fruits

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Abstract

Postharvest diseases cause considerable losses to harvested fruits during transportation and storage. Decays in fruits storage and transportation mostly caused by fungal and bacteria. These organisms may cause soft spots or light brown lesions on fruit. Fungal growth, in a variety of colors, may also be apparent on the surface of infected produce. In time, the entire fruit or vegetable can become dry and mummified, or, under moist conditions, a soft, wet mass. Previous research explain that microbial infection in the fruit may start before or after harvesting. Synthetic fungicides are primarily used to control postharvest decay loss. However, the recent trend is shifting toward safer and more eco-friendly alternatives for the control of postharvest decays. One of the potential material control of postharvest decays is liquid smoke coconut shell. Liquid smoke, is a condensed smoke from pirolyzed coconut shell. The condensed smoke delivers an ability in preserving food due to the presence of acids, carbonyl and phenolic compounds. Previous research showed that liquid smoke had antimicrobial effect for fungal and bacteria. Therefore liquid smoke has potential for use as an all-natural antimicrobial for extending the shelf life of horticultural product.

Keywords: Liquid smoke, Fruit, Antimicrobial, Fruit decays

1. Introduction

Fruits are perishable food product. Fruits are alive and has to stay alive long after harvest. Physiological activity has become problem in the storage and caused the short shelf life. The other problem in the maintain shelf life fruits is microbial attack or pathogenic attack. Microorganisms readily attack fresh produce and spread rapidly, owing to the lack of natural defense mechanisms in the tissues of fresh produce, and the abundance of nutrients and moisture which supports their growth. Microorganism or pathogen infected could begin in the field or after harvesting. The early symptom in the fruit damage often been appeared after harvest, in the storage condition or after the fruit is ripe.

Superior fruits in Indonesia, such as zalacca and mango, microbial attack becomes main problem in the storage and distribution and need to control. Previous research showed that symptom in zalacca fruits begin with the black spot in the corner of the fruit. Microbial attack in mango fruit could be caused by Colletotrichum sp. It is important to control microbial attack in the fruits along storage and distribution, because it was affecting for the expanding market of the fruits. The high levels of decay caused by fungal pathogens can be directly attributable to the large amount of nutrients and water, low pH, and the decrease in intrinsic decay resistance of fruit after harvest (Liu et al. 2013).

Control of postharvest decay is becoming a difficult task, since the number of pesticides available is rapidly declining as consumer concern for food safety is increased. Liquid smoke,

a condensation product of coconut shell pyrolysis, could be suggested for being an alternative solution to overcome the problem. The liquefied smoke has been developed as food preservative agent, food antioxidant and biopesticide (Yuningsih and Anggraeni, 2013). Traditionally, smoking has been used for the food preservative.

This paper will study about the potential of liquid smoke coconut shell in extending the shelf life of tropical fruits especially for the superior fruits in Indonesia.

2. Postharvest diseases of superior tropical fruits in Indonesia

Diseases fruits especially tropical fruits mostly caused by fungal, or microbial attack. This microbial attack caused by pathogens are primarily field diseases, and often accelerate after harvest.

One of the superior fruits in Indonesia with high sensitivity with microbial attack is zalacca fruit. In zalaaca fruits, early attack of microorganism is in the corner of the fruits. Decay in the zalacca fruits began by the black spot in the corner continued with mycelia growth and the fruits became watery and soft (figure 1). The microbial attack became the main problem in extending shelf life in zalacca fruits. In the packaging system (modification atmosphere packaging/MAP), infected fruits triggered the damage of the other (Yulianingsih et al. 2010).

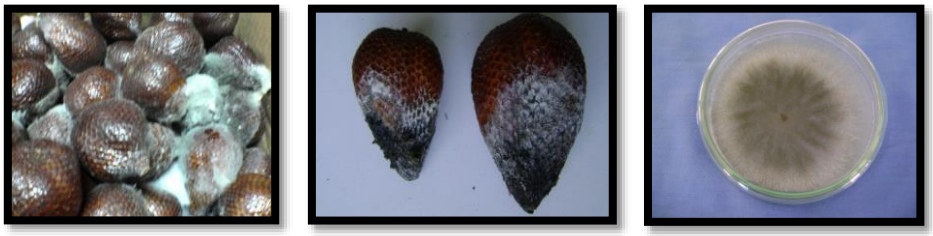

Figure 1. Microbial attack in zalacca fruits (Yulianingsih et al. 2010)

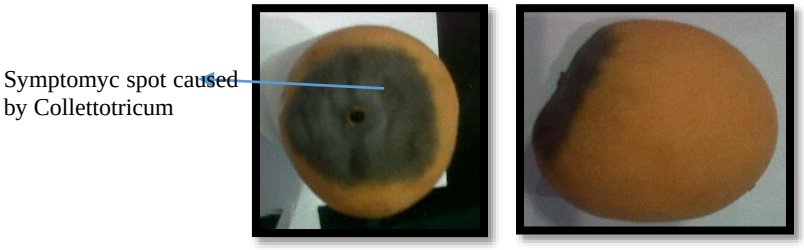

Figure 2. Colletotrichum sp in mango fruits (Mulyawanti et al. 2011)

In the mango fruits, microbial attack mostly caused by fungal *Colletotrichum sp* (Figure 2.). Other research explained that decay in mango fruit caused by Aspergillus niger, Pennicillium expansum, Alternaria alternata, and Botryiospaeria sp. Tropical fruit decay such as papaya and pineapple were also caused by fungal. Market diseases pathogens detection of important fruits in Shanghai showed that the larger products found in the market were bananas, grapes, apples, and oranges. The diseases of the fruits mostly caused by fungal, and the identified fungals were *Alternaria sp*, *Penicillium sp*, *Fusarium sp*, and *Botrytis sp* (Teng-Fei et al. 2009).

3. The potential of liquid smokes as antimicrobial/antifungal

Coconut shell is one of coconut parts and it is by product of coconut processing. Liquid smoke coconut shell is a condensed smoke from pirolyzed coconut shell. Coconut shell belongs

to hard wood group that containing three main components: cellulose, hemicelluloses, and lignin. Cellulose decomposition by heat results in anhydroglucose, carbonyl compound and furan. Decomposition of hemicellulose is similar to that of cellulose, but resulting in acetate acid and carbon dioxide (Lombok et al. 2014).

Traditionally, liquid smokes has been used for preservation foods. In the traditional shallot storage, smoking maintain the temperature of storage room and also decreased microbial damage along storage.

Liquid smoke is high content of polyphenol (Table 1.). Result of GC-MS analysis showed that liquid smoke coconut shell contain phenols, aldehyde, ketones, and organic acid compounds. Three main components of liquid smoke (phenol, carbonyl and organic acid) are able to suppress the growth of fungi that cause a decrease in the quality of food (Soedijo, et al. 2015). Acid compounds together with phenol and carbonyl synergically act as antimicrobial. The most acid compounds contained in liquid smoke are derivations of carboxylate acid such as furfural, furan, acetate acid, propionate acid, butyric acid and valerate acid (Santoso RS, 2016). Antifungal effect of coconut shell pyrolytic oil against wood decay fungi (Shiny et al. 2013).

Table 1. Volatile compound from liquid smoke coconut shell

Compound	Retention Time
Acetic acid	2.8918
Hydrazine, ethyl-	3.2578
Pyridine	4.3086
Phenol	7.9932
1-Buten-3-yne, 2-methyl	8.2343
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	9.6452
Phenol, 2-methyl-	9.8223
Phenol, 4-methyl	10.6251
Phenol, 4-methoxy	10.7255
Phenol, 2-methoxy	10.9557
Phenol, 2,4-dimethyl-	12,3724
Phenol, 3-ethyl-	13.0868
2-Methoxy-5-methylphenol	13.5886
Phenol, 2-methoxy-4-methyl-	13.7893
Phenol, 4-ethyl-2-methoxy-	16.1801
Phenol, 2,6-dimethoxy	18.1755
Phenol, 2-methoxy-4-propyl	18.4175
Methyl p-anisate	18.6418
4-Methoxy-2-methyl-1-(methylthio)benzene	20.3833

Phenols, carbonyls, and organic acids significantly contribute as antimicrobial potential. Previous research showed that polyphenol has ability to disturb microbial growth so that potential to be preservative agent and extending shelf life the fruits. Phenolic compound can disturb cytoplasmic membranes and cause intracellular fluids in microorganism to leak. Carbonyls inhibit microbial growth by penetrating the cell wall and inactivating enzymes located in the cytoplasm and the cytoplasmic membrane. Antimicrobial potential of organic acids is accredited to the influence on overall pH and the undissociated form of the acid. The cell membrane lipid bilayer can be easily penetrated by organic acids in their undissociated forms. Because the pH inside the cell is higher than the exterior, the acid is highly dissociated inside the cell. Once dissociated inside the cell, the cell will deplete all of its ATP reserve

energy transporting the dissociated protons out of the cell. This leaves the cell unable to perform essential metabolic pathways needed to sustain life (Milly, 2003).

4. Conclusion

Decays in fruits storage and transportation mostly caused by fungal. Fungal growth can be disturbed by phenol. Liquid smoke coconut shell rich of phenol compound and because of that liquid smoke coconut shell is potential in extending shelf life of fruits.

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The Effects of The Application of Edible Coating, Antimicrobial Agent, Packaging and Absorber on Snake Fruit (*Salacca edualis* REINW)

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Abstract

Snake fruit or salacca fruit (*Salacca edualis* Reinw) is a non climacteric fruit, very perishable thus has a short shelf-life. The main problems in the storage of snake fruit are microbia infestation, the growth of fungal mycelia on the skin, the flesh becomes brownish, moist and even watery, or sometimes the skin becomes dry and tough, making it hard to peel. Therefore, a proper handling technology is needed to extend the shelf-life and maintain the quality of snake fruit. The technology should be able to lower the respiration rate or delay an early maturation, and prevent physical and microbiology deterioration. Previous studies had reported the positive effects of low temperature, edible coating, antimicrobial agents, packaging and absorber to the shelf-life of fruits. The objective of this research is to study the effects of the combination of those treatments to the quality alteration of snake fruit. Treatments being applied were edible coating (nanochitosan), antimicrobial agent (galangal extract), packaging material (low density poly etylene/LDPE bag and plastic-wrapped styrofoam tray), and absorber (nanozeolite). Snake fruits were kept in 12oC cold storage and analysis was done every week for two weeks. Parameters being observed were weight loss, colour, pH and total soluble solid. The results showed that snake fruit coated with nanochitosan and galangal extract, packed in LDPE bag and using nanozeolite had the better quality compared with other treatments.

Keywords: snake fruit, packaging, edible coating, antimicrobial agent, absorber

1. Introduction

Snake fruit is a non climacteric fruit, very perishable, thus has a short shelf-life. Worsened by the hot and humid of tropical climate, the shelf-life of snake fruit is even shorter (Manurung *et al.* 2013), i.e. approximately 7 days in room temperature. The high water content (78%) and carbohydrate content (20.9%) may cause snake fruit rot easily when stored in room temperature (Kosenda, 2005). A certain handling effort to extend the shelf-life and preserve the quality of snake fruit is needed. Previous researches had reported extended shelf-life of snake fruit by lowering the rate of respiration, delaying early maturation and preventing physical and microbiological defect, therefore the freshness of the fruit can be maintained at an acceptable level for consumers.

Storage in low temperature has been proved to be able to extend the shelf-life of snake fruit. The longest shelf-life was obtained with storage at 3-5^{o}C (25 days), or 7-10^{o}C (23 days) (Mahendra *et al*. 2013). However, both cases showed moderate to severe chilling injury problems. Although these treatments gave better result compared to room temperature storage, chilling injury is a concern that should be considered serious. Fruit with chilling injury is not acceptable by the consumers. Storing snake fruit at 15^{o}C only extended shelf-life for 2.5 days

without causing chilling injury. Packing snake fruit in polyethylene bags is one of the most effective way to reduce the incidence of chilling injury in 5-10^{o}C storage. In addition, it can also increase the shelf-life from 18 days to 26 days in 10^{o}C storage. Other postharvest handling technology for snake fruit aside from storage and packing treatments need to be studied in order to optimized the improvement of shelf-life.

An established technology to lower the respiration rate is edible coating. Edible coating is commonly applied to improve the appearance and resilience of food due to its environment friendly properties. Edible coating works as a barrier to humidity and oxygen during handling, processing and storage, thus prevent the deterioration of food. Moreover, it can also increase the safety due to its natural biocide activity or antimicrobia agents (Hassanpour, 2015).

Similar with other fruit in general, snake fruit has a natural layer on the surface which cover the pores, thus reduce the resporation and transpiration rate. During postharvest handling, parts of this layer might be removed in the process of washing, rubbing, or friction and impact happened during transportation. This may cause dull-looking of the fruit (Kosenda, 2005). To replace the natural layer, a wax layer coating may be applied (waxing) as postharvest treatment.

The main damage occured in snake fruit during storage is caused by fungi infestation, where the fungal mycelia grows on the skin, the color of the flesh becomes brownish, soft, watery and even rotten, or the skin becomes dry and hard, makin it hard to peel. The utilization of proper antimicrobial agent may help inhibit the infestation of microorganisms and prolong the fruit shelf-life. Galanga (*Alpinia galanga* L. Swartz) is a kind of herb from *Zingiberaceae* family which is known to be useful as natural antimicrobial agent (Arbie, 2010).

2. Method

Snake fruit was obtained from Snake Fruit Farmer Association (Sleman, DI Yogyakarta, Indonesia) and transported to Bogor, West Java, Indonesia using air and land transportation. Application of treatments and analysis was carried out at Research and Development Laboratory, Indonesian Center for Agricultural Postharvest Research and Development, Bogor, Indonesia.

The application of active packaging for snake fruit was conducted through these steps: snake fruit was sorted and graded according to the standards for export purpose, prior to dry cleaning. Afterwards, coating agent (nanochitosan) with and without antimicrobial agent (galangal extract) was applied and snake fruit was air dried before packed in two different packaging types, i.e. LDPE plastic bags and plastic-wrapped styrofoam tray. Pores were made on the LDPE bags by pricking the plastic with needle. Each package contained 500 grams of snake fruit. Nanozeolite (in tea bag) was inserted to half of the packages. The samples was then stored in cold room at 12oC.

The effects of coating, packaging and nanozeolit application were studied. Observation and analysis was done every 7 days for 2 weeks. Parameters being observed were weight loss, colour (Chromameter), pH (pHmeter) and total soluble solid (Refractometer).

3. Results and Discussion

a. Weight loss

The observation on weight loss during the two weeks storage (Table 1) showed that snake fruit without any treatments experienced the highest weight loss. Between the treated snake fruit, the increase of weight loss from week 1 to week 2 was found more on snake fruit packed

in crates, even with coating and galangal extract. LDPE showed better influence for weight loss compared to styrofoam tray with plastic wrap. The weight loss of snake fruit observed in the treatments with or without nanozeolite was inconsistent. Snake fruit with nanozeolite did not show lower weight loss, whether the snake fruit was packed in LDPE or plastic-wrapped styrofoam tray, and coated or uncoated.

Snake fruit experienced weight loss in the first days after harvest (Santosa *et al*. 2016). It was observed that 1-20% weight loss in their study with the variation of treatment using chitosan coating and storage temperature. Snake fruit coated with chitosan and stored in 15^{o}C storage had 24 days of shelf-life. Other research reported that snake fruit with galangal extract coating experienced weight loss of 1,24% after 21 days storage in 15^{o}C (Arbie, 2010).

Table 1. Weight loss, pH and TSS changes of snake fruit during storage (12^{o}C)

Treatments		Weight loss (%)	pH	Total soluble solid (^{o}Brix)
Control	Week 1	11.01	4.56	15.70
	Week 2	8.10	4.35	14.10
LDPE only	Week 1	0.39	4.47	13.35
	Week 2	1.61	4.49	17.20
Crates+nanochitosan+galangal extract	Week 1	6.97	4.34	13.10
	Week 2	10.74	4.52	17.05
LDPE+ nanochitosan+galangal extract	Week 1	0.41	4.27	12.25
	Week 2	0.69	4.37	12.70
LDPE+nanozeolite	Week 1	0.53	4.37	12.75
	Week 2	1.12	Rotten	rotten
LDPE+nanochitosan+ galangal extract+nanozeolite	Week 1	0.74	4.35	12.70
	Week 2	0.90	4.29	15.20
Plastic-wrapped styrofoam tray+ nanozeolite	Week 1	2.84	4.42	14.85
	Week 2	4.18	4.32	17.00
Plastic-wrapped styrofoam tray+ nanochitosan+galangal extract +nanozeolite	Week 1	2.44	4.49	14.90
	Week 2	3.75	4.37	16.20

The loss of weight that occures is directly proportional with transpiration and respiration. Respiration can cause weight loss due to the burning of sugar and other substrate such as fat and protein that are converted into CO2, water vapor, and energy. The byproduct of respiration in the form of gas will evaporate (Wills *et al*. 1981). Transpiration process is the loss of water due to evaporation. High rate of evaporation may be caused by the difference of water pressure outside and inside the snake fruit. The water pressure inside the fruit is higher than outside, thus water vapor will break out and causing the loss of water content of the fruit, showed by the loss of weight.

Based on the packaging materials, the study results showed that LDPE bags with pores are more effective in controlling weight loss during storage. Styrofoam, even with the addition of nanozeolite bags failed to suppress the weight loss of snake fruit. Porous LDPE bags may increase the rate of air exchange in the packaging and functions as barrier to CO2, O2 and water. Unpacked and uncoated snake fruit can undergo rapid respiration and water loss. The evaporation may be even more rapid and causing the water contained in cells or in between cells increased, thus water will be released into the air and cell will lose water. Further, the environment humidity will increase and promote the infestation of microorganism (Arbie, 2010).

Horticultural products (fruit and vegetable) are considered not suitable for sell if the weight loss were 5-10% (Pantastico, 1975). Based on this parameter, the LDPE packed snake fruit in this research was suitable for sell.

b. pH

Analysis of pH of snake fruit is shown in Table 1. There was no significant changes of pH in all the treatments. Mahendra *et. al*. (2013) reported the same conclusion from their research where after 7 days of storage, the quality measurement to snake fruit acidity showed no influence from different packaging and coating treatments.

Climacteric fruits experience sugar content increment and acidity reduction during storage, while in non climacteric fruits such as snake fruit, the pH only changes slightly. Most fruits and vegetables constantly undergo organic acid metabolic alteration. Total acid of fruits decrease during ripening process although some acids may increase. Starch hydrolyzes into simple sugars and further converted into organic acids, causing the increase of total acid of snake fruit. The organic acids can also be formed from protein and sugar degradation when the respiration process is undergoing. The existing microbia can also play part in the increase of total acid in snake fruit due to its acid production during metabolic activity (Tranggono and Sutardi, 1989).

c. Total soluble solid (TSS)

Almost all treatmenst in this study showed the increasing of TSS during storage. Reduction of TSS was found only in the control sample. This may be caused by the respiration process producing piruvate acid and CO2 and H2O from sugar (Santosa *et al*. 2016). During the ripening process, the solid was hydrolyzed into sucrose and converted back causing the decrease of sugar content while respiration process proceeded. The common occurrence during storage is, sugar content increases and then decreases, which trend is in accordance with the pattern of respiration process. Therefore, TSS will fluctuate during storage. This explains the data obtained in this study where uncoated and unpacked snake fruit showed faster reduction of sugar content compared to other treatments, based on the TSS value (Wills *et al.* 1981).

This fact confirms the statement of Arbie (2010), that snake fruit's TSS increased after the fifth days of storage and decreased after the tenth days. The increase of TSS was caused by the breakdown of complex components, eg. carbohydrate polymers especially starch, into sucrose, glucose and fructose. These simple components are easily soluble in water. The decrease of TSS was caused by the utilization of simple sugars as substrate in the respiration process (Paramawati *et al*. 1998).

In the course of respiration process, there are three phase, i.e. the breakdown of polysaccharides into simple sugars, causing the increase of sugar content; followed by the oxidation of simple sugars, producing piruvate acid and other organic acids, causing the

decrease of sugar content; and ended by the transformation of piruvate and other organic acids aerobically into CO2, H2O and energy, until the organic acids were significantly reduced (Pantastico *et al*. 1975). In the ripening period the sugar content increases, and in the aging period it decreases (Tranggono and Sutardi, 1989).

d. Colour

Colour is one of the important factors in assessing snake fruit quality, both of the skin and the flesh. The observation showed that colour alteration occured more in unpacked samples compared with those packed in LDPE bags and plastic-wrapped styrofoam trays (Table 2).

Table 2. Colour alteration of snake fruit during storage (12^{o}C)

Treatments		Skin			Flesh		
		L	a	b	L	a	B
Control	Week 1	33.57	9.69	14.52	82.29	-3.63	25.08
	Week 2	26.51	12.56	16.52	74.43	-2.45	24.24
LDPE only	Week 1	30.11	14.29	24.37	81.19	-2.84	26.30
	Week 2	38.90	10.38	13.79	80.62	-4.91	23.29
Crates+nanochitosan+galangal extract	Week 1	33.96	8.47	10.72	82.58	-2.88	24.54
	Week 2	27.26	11.79	14.51	75.05	-0.02	23.47
LDPE+ nanochitosan+galangal extract	Week 1	25,13	15,23	24,41	81,91	-2,65	24,93
	Week 2	34,75	8,12	10,93	82,04	-4,28	23,59
LDPE+nanozeolite	Week 1	28,62	13,83	23,73	80,20	-2,68	25,92
	Week 2	36,43	7,02	11,47	78,23	-3,15	23,06
LDPE+nanochitosan+ galangal extract+nanozeolite	Week 1	27.75	14.98	24.69	81.32	-2.32	24.89
	Week 2	34.33	6.41	9.36	81.21	-3.01	21.67
Plastic-wrapped styrofoam tray+ nanozeolite	Week 1	30,92	13,88	24,36	81,55	-2,89	26,70
	Week 2	36,40	7,69	10,76	69,97	0,01	20,23
Plastic-wrapped styrofoam tray+ nanochitosan+galangal extract +nanozeolite	Week 1	30.25	15.44	23.29	82.66	-2.78	25.32
	Week 2	31.76	12.65	14.82	68.57	-0.53	24.09

The colour of snake fruit skin alters from dark to lighter during ripening process (Figure 1), which is caused by carotenoid pigment. Basically, there are two types of carotenoid, i.e. β-carotene and xanthophyll. β-carotene causes red colour of the fruit, while xanthopyll gives yellow colour. During the ripening process, the amount of xanthopyll will decrease and the amount of β-carotene will increase (Winarno, 2002). Carotenoid is a stable compound and will stay in the tissue even when senescence occurs.

Figure 1. Unripe and ripe snake fruit

The colour of snake fruit's flesh altered to grayish or brownish during storage. The browning process initially occured on a bruised or injured tips, usually happened when skin is peeled, then the browning spreaded to rest of the flesh. The browning parts of the flesh were not as compact as in the other parts, and sometimes the cells were open. When a part of the skin was opened or peeled, the contact with air (oxygen) was more extensive, thus the activity of phenolase enzyme was higher. The brown colour was developed from the enzymatic browning reaction due to oxydation. Snake fruit contained compounds such as polyphenol in the form of tannins. The browning reaction which occurs when oxygen made contact with polyphenol, catalyzed by polyphenol oxidase, forming melanin compound, which has brown colour. Oxygen can make contact with polyphenol when cell or tissue is open upon an injury (Winarno, 2002).

The browning condition on the flesh pf snake fruit skin may be caused by polyphenol oxidase or phenolase enzymes. Polyphenol oxidase enzyme (PPO) with the help from oxygen will transform monophenol group into O-hydroxyphenol, which further will be converted into O-quinone. This O-quinone group is the cause of the brown colour appearance (Tranggono and Sutardi, 1989).

A brown spot, round with unclear or blur borders on the skin of snake fruit (Figure 2) is a symptom of rotting fruit caused by *Fusarium sp*. This spot is easily spread and the flesh underneath it will rot immediately. On the rotten and brown skin, white fungal mycelium will form, cotton-looking (Figure 3), and can cover the whole surface in 6-7 days (Martoredjo, 2009).

Figure 2. Dark brown spot on snake fruit skin

Figure 3. White fungal mycelium on snake fruit skin

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Packaging Design and Postharvest Treatment to Maintain the Quality of Rambutan (*Nephelium Lappaceum* L.) in Distribution System

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Abstract

Handling of rambutan distribution need to be improved, due to rambutan has a potential in export markets. In maintaining the quality of rambutan during distribution depends on the interrelationships between it's packaging and postharvest treatment. The purpose of this research was to design packaging using corrugated board that combined to perforated plastic bag and coating treatment of rambutan using aloe vera L. Packaging was designed by using flute BC of corrugated board with RSC (Regular Sloted Container) packaging type. 12 kg of rambutans were packed in two perforated plastic bags (polypropilene). Each bag has wide totally holes 4.13% from surface area. Before packaging, rambutans were deeped for 30 seconds in 20% concentred *Aloe vera* L. solution as a coating treatment. Rambutan were packaged in cardboard without plastic bag as a control. Packaging design for 12 kg of rambutan was 540 x 360 x 200 mm with partition in the middle of the box. After transportation using the simulator (frequency: 3.02 Hz; amplitude: 2.78 cm), which is similar to 144.14 km, the mechanical damage of rambutans were 3.75% for control and 1.11% for treatment. After ten days stored at 10^{o}C, the total damage of rambutans were 44.17% for control and 38.33% for treatment. Rambutan with aloe vera L. coating treatment was still acceptable for consumers till 10 days. The acceptance of consumers based on organoleptic test for peel freshness, peel color, and flesh of fruit taste.

1. Introduction

Rambutan production in Indonesia increases continuously. BPS data (2015) shows: 522.852 ton (80.492 ha) in 2010 become 737.239 ton (102.843 ha) in 2014, so it takes a market expansion, both domestic and export markets. On export markets, quality of rambutan Indonesia has not been able to compete with other producers such as Thailand, the Philippines, and Australia (Margono 2009). The weakness of rambutan is easily wither represented by the color brown and desiccate of rambutan peel. This is due to a high transpiration from peel of rambutan which have many stomata (O'Hare *et al*. 1994). Several studies have been done to preserve the freshness of rambutan, including coating applications with *Aloe vera* L. combined with PP perforated plastic bag packaging (Darmawati *et al.* 2016) and the application of silica gel combined with PE plastic bags by micro-perforations (Maulita 2015). Post-harvest treatment also need to be done is to improve the distribution system of rambutan using appropriate packaging to protect it's as long as transportation and distribution process. Currently, the distribution of rambutan is generally

performed in the form of bonds and laid out directly on a flatbed of pickup (truck). Corrugated cardboard is a commonly used as a fruits and vegetables packaging, because carton is flexible so that can be designed in various shapes and sizes. Another advantage of corrugated board is having a smooth surface and capability in reducing of vibration, so that can reduces the risk of mechanical damage. Purpose of this research was to design a distribution packaging made from corrugated board combined with post-harvest treatment to protect the mechanical and physiological damage of rambutan during transportation and distribution.

2. Materials and Methods

Rambutans (Lebak variety) were harvested from farmer orchard at Cileungsi-Bogor. The level maturity of rambutans ware 85-100% (peel the fruit is red or orange), with a diameter of 4.69 ± 0.46 cm and weight of 31.59 ± 2.61 g. *Aloe vera* L. as a coating material were obtained from Parung-Bogor, corrugated cardboard flute BC and plastic bag (polypropylene) as a package material. The apparatus were simulator table for transport simulation, cold storage, refractometer for measure the total soluble solid.

2.1 Design Packaging

Packaging was designed as a box-shaped (RSC type) with BC flute material. Before a packed, rambutan were coated by *aloe vera* L. solution with a concentration of 20% and packed in plastic bags perforations (Rusnaldi 2015). Weight per package was 12 kg and were divided into two plastic bags, each weighing 6 kg per bag. The purpose of this treatment was to minimize of water vapor trapped on the surface of the plastic during storage (Heriansyah 2014).

2.2 Determined dimension of package box (RSC type)

Inner dimension of package was determined based on the volume of rambutans in which the total weight per package was 12 kg. Calculation of volume and the number of rambutan per package was used equation 1 and 2.

$$J = J_b \times K_k \quad (1)$$

$$V_b = J \times V_{ib} \quad (2)$$

Where : J= number of rambutan in package; J_b= number of rambutan in each kg ; K_k= weight of rambutan per package (kg); V_b = Volume total of rambutan per package; V_{ib}= volume of each rambutan

Inner dimension was determined using simulation by combining the value of Pi, Li and Ti corresponding to the volume of fruit (Vb) with ratio of Pi and Li over 0.5 and accordance with pallet size that used in the distribution process. Equation 3-9 ware determined of inner, design and outer dimension of box package.

$$V_b = P_i \times L_i \times T_i \quad (3)$$

$$P_d = P_i + 2 \times df \quad (4)$$

$$L_d = L_i + df \quad (5)$$

$$T_d = T_i + df \quad (6)$$

$$P_k = P_d + 2 \times df \quad (7)$$

$$L_k = L_d + df \quad (8)$$

$$T_k = T_d + 2 \times df \quad (9)$$

Where P =Length (mm), L =Wide (mm), T =high (mm), df = thickness of *flute* BC (mm); subscribe i, d and k were inner, design, outer dimension of box respectively

2.3 Preparation of Rambutan

Rambutan was cleaned of branches and leaves using scissors and sorted according to the level of maturity and diameter size. Rambutan has been sorted was weighed as much as 58 kg and were divided into two parts for the treatment (coating and packing in perforated plastic bags pp) and control (without coating and plastic bags). Rambutan was treated (PPAV), it were washed and dipped in a solution of *Aloe vera* L. with a concentration of 20% for 30 seconds and than the wind dried. Rambutans were weighed 6 kg and put into plastic bags perforated and put it in a cardboard box, each box contains two bags (2 x 6 kg). As a control (NKP), rambutans were packed in boxes weighing 12 kg without coating treatment and plastic bags.

2.4 Simulation of Transportation

To find out the strength of packaging, transport test was done using a table simulator with frequency of 3.02 Hz, amplitude 27.8 mm for 3 hours, equivalent as distance 144.14 km of road. After the simulation, rambutan stored at a temperature of 10°C for 16 days and measured the amount of damage, peel color and total soluble solids. Measurements and observations conducted every two days. Organoleptic tests were conducted to determine the freshness of the fruit (fruit color) which was accepted by the consumer.

2.5 Mechanical and physiological damage

Mechanical damage was observed visually by looking of the discolored or darker brown color that appear at the peel surface of rambutan. The observation was done after a day simulation of transportation because generally the damage will be seen. Physical and chemical changes during storage were observed and measured every two days.

2.6 Total soluble solid (TSS)

The measurement of TSS conducted by digital refractometer. Rambutan juice is placed on the refractometer prism and the results showed the levels of TSS (^{o}Brix).

2.7 Measurement of peel color of rambutan

Skin color represents the freshness of rambutan. The method of measurement the color of rambutan was used image processing system (image processing). The result measurement were the value of Red, Green and Blue (RGB), that the value was processed into value of a and b. Values of a and b was processed into degree of Hue value. Degree of Hue value was obtained from equation 10 (Concellon 2007):

$$^{o}Hue = \tan^{-1} b/a \qquad (10)$$

Where a: red-green color value, b: yellow-blue color value

2.8 Organoleptic test

Organoleptic test was conducted to determine the level of consumer preferences on rambutan that has been transported and stored at a temperature of 10°C. The test was done by 25 panelists using a scoring 1 (strongly dislike), 2 (do not like), 3 (somewhat like), 4 (neutral), 5 (a bit like), 6 (like), and 7 (most like), value 4 was used as the minimum limit of panelists accepted.

3. Result and Discussion

3.1 Package Dimension

Weight average of rambutan sample was 31.59 ± 2.61 g with a volume of 28.6 ml (density of rambutanis 1104 kg / m3). The volume of rambutan in package with has weight 12 kg per package was 12.01 ml and the number its 420 pieces. Outer dimensions of packaging which calculated based on the volume of fruit in package was obtained 540 x 360 x 200 mm. The package can be arranged on a pallet size of 1100 x 1100 mm, with the configuration of 2-length way and 3-wide way (key 5 arrangement). Dimensional design was shown in Figure 1, while the package product was shown in Figure 2. Ventilation was added on the package with total area 2% from surface area of the package. Ventilation has an important role to maintain the quality of the fruit in the package. Air circulation in package will affect the quality of the fruit package (Singh J *et al*. 2008; Saeed *et al*. 2010; Darmawati *et al*. 2010).

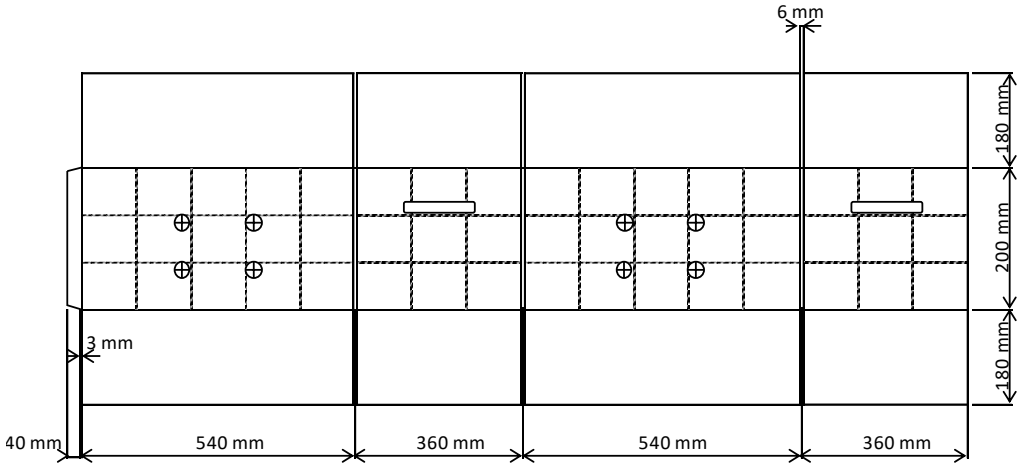

Figure 1 The planned packaging design

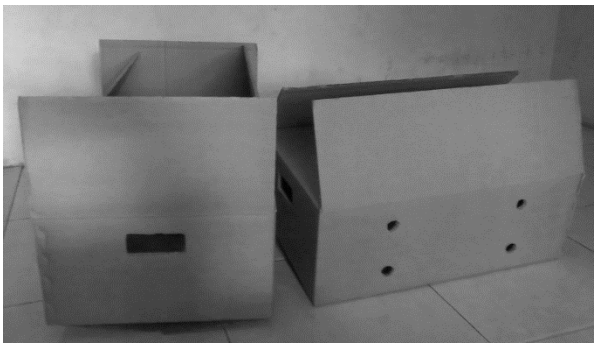

Figure 2 The research packaging design

3.2 Perforation of plastic bag

The purpose of the using plastic bags is to reduce the rate of rambutan transpiration. If the plastic bag is not given perforations, it will cause the water vapor as a result of respiration was trapped on the surface of the plastic and become into the water droplets. Whereas if not using plastic bags, rambutan will be quick dried (Heriansyah 2014). Rusnaldi (2015) was improved the previous studies by using perforated plastic. Based on these results, this research was conducted by giving perforation of pp plastic bag with a size of 8 mm by 40 is equivalent to 4.13% of the surface area of plastic bags.

3.3 Mechanical damage after transportation

Transportation was conducted using simulation table with frequency of 3.02 Hz, amplitude 27.8 mm for 3 hours, equivalent as length 144.14 km of road. Mechanical damage after a day transportation was 1.11 % for PPAV (treatment) and 3.75 % for NKP (control). Vibration from the simulation table has vertical direction that was represented the vibration during transportation by truck (Nugroho *et al.* 2011). Vibration during transportation will be affect the decrease of fruit quality. The mechanical damage of rambutan can accelerate the change of peel color and accelerate wilting of fruit (Landrigan *et al*. 1996). The results of the study Jung and Park (2012) showed that vibration during transport was accelerated the degradation of apple quality, increased of weight loss, ethylene production and decreased the hardness of fruit.

3.4 Physiological change during storage

Result of the observation and measurement amount of rambutan damaged after transport and stored at a temperature of 10°C was presented Figure 3. After ten days in cold storage (10ºC), the damage of rambutan treatment (PPAV) was 38.33%. This value is lower than the control, which reached 44.17%. Rambutans were packed in a perforated plastic bag and coated with *Aloe vera* L. were seen fresher then the control. Peel of rambutan as control looks dry and chapped (Figure 4). These results indicate that *Aloe vera* L. coating can inhibit the transpiration, so the rambutan still looks fresh, while the perforation of bag has not been able to minimize the moisture trapped on the surface of the plastic packaging. That it was shown by the many water droplets on the surface of a plastic bag and a lot of rambutan looks damp and rotten.

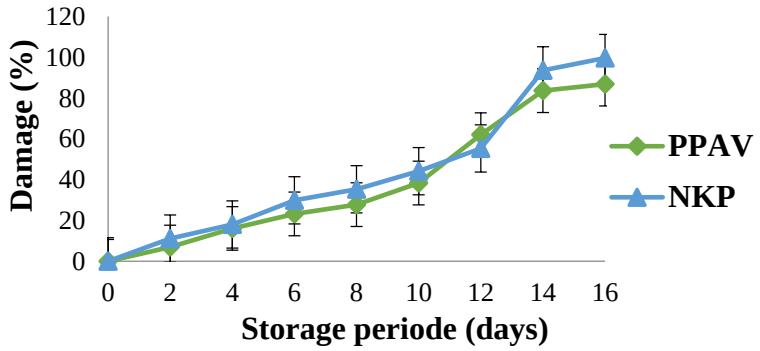

Figure 3 Percentage of the amount of damaged rambutan during storage at 10°C

Figure 4 Physical changes of rambutan on day 10 (a) PPAV packaging; (b) NKP packaging

3.5 Total Soluble Solid (TSS)

Rambutan is non klimateric fruit, so that the ripening after post-harvest does not occur. This was showed by declining of TSS during storage. TSS is still high until 10 day of storage, then decreased until day 14 (Figure 5). The graph was showed that the treated of rambutan has a TSS was higher in day 8 of storage and it has indications that the treatment can be maintain the sweet taste of fruit than control.

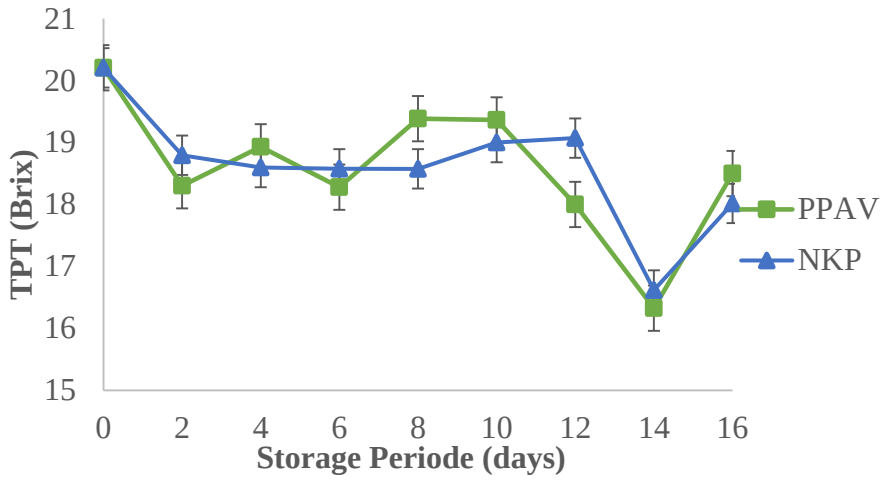

Figure 5 Change TSS value of rambutan during storage at 10°C

3.7 Color of peel

The color of rambutan during storage was changed from red to dark. At the start of storage, the value of hue was 53.21 and at the end of the storage becomes 40.98 for PPAV treatment and 38.97 for control (Figure 6). The color of rambutan untreated (control) were quickly turns into a dark, fruit peel and hair become dry and lightly browned (Figure 4). This indicates that the treatment able to inhibit the change of color or freshness of rambutan.

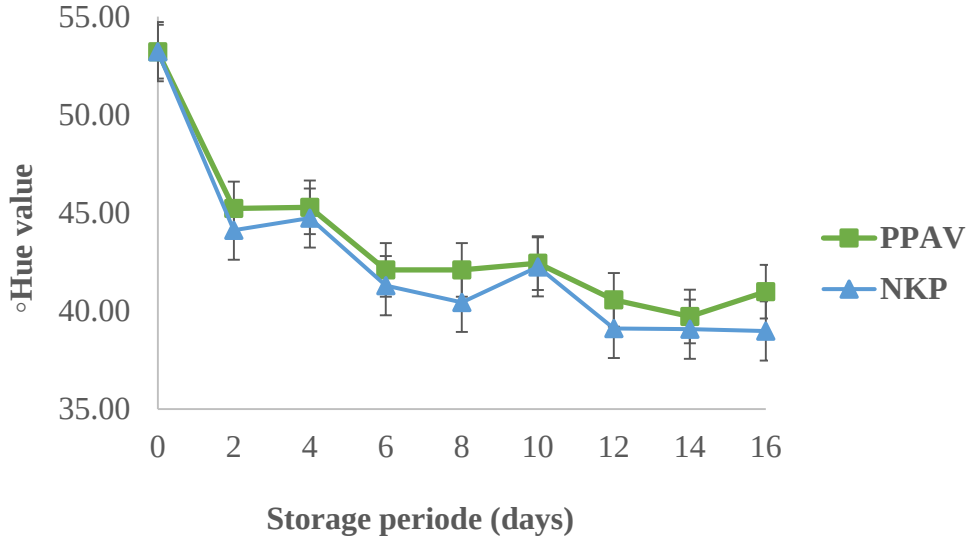

Figure 6 Change in ◦hue value color of peel during storage at 10°C

3.8 Organoleptic test

Peel color and freshness of rambutan is an important thing that appeals to consumers. Result of organoleptic test showed a rambutan treated was still acceptable by panelist until 10 days of storage, which two day later than control based on color or freshness of rambutan and taste of flash (Figure 7 and Figure 8) that means the result of treatment better than control.

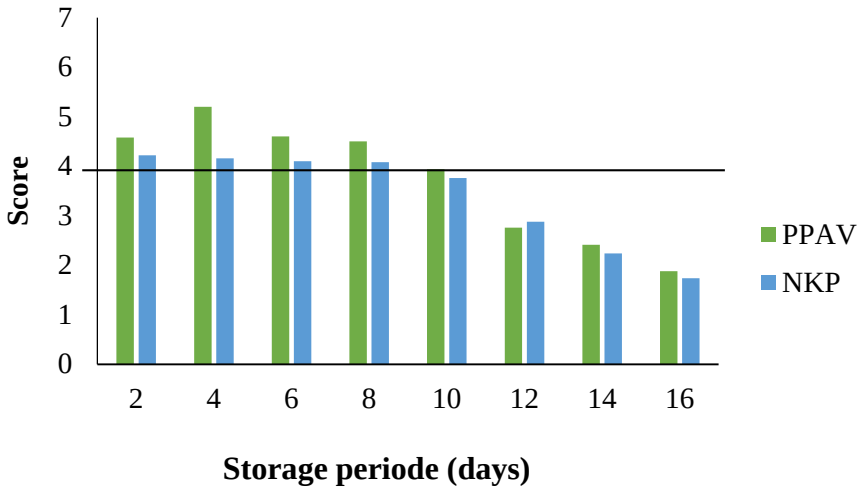

Figure 7 Assessment of panelist for peel freshness during storage 16 days

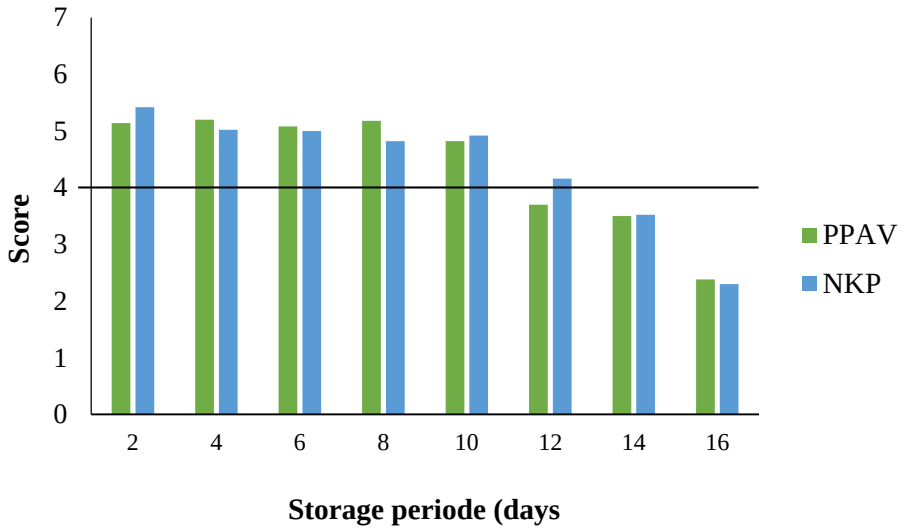

Figure 8 Assessment of panelist for flash taste during storage 16 days

4. Conclussion

Packaging systems and post-harvest treatment coating using a solution of *Aloe vera* L. concentration of 20% were able to maintain the freshness of rambutan and still be accepted by the panelists until 10 days of storage at temperature of 10°C. The packaging system was consists of a cardboard box (RSC type) from flute BC, dimensions 540 x 360 x 200 mm and two perforated plastic pp bags, weight of each bag was 6 kg of rambutan, total capacity per carton board box was 12 kg. The mechanical damage after a day transportation was 1.11% and increased become 38.33% after 8 days storage period. This result better than control which itswere 3.75% and 44.17% respectively.

5. Recomendation

Need further studies to determine the number and position of the perforation holes in real scale of bags was used in the field (scale up of laboratory results).

Packaging dimensions need to be redesigned in order to obtain the optimum size for rambutan that has a high respiration rate (eg determining the height of the packaging which minimizes the moisture trapped in the pile).

Reviewing the applications of coating using spray method, caused a rambutan surface was fully with hair.

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Characterization of Local Durian Varieties in Central Java Using Molecular Markers Inter Simple Sequence Repeats (ISSR)

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Abstract

The lack of morphological markers encourages the development of other markers associated with material controlling an individual characterize, known as DNA molecular markers. Inter simple sequence repeat (ISSR) can reveal a plant diversity. The research aims are to identify the genetic diversity and determine the identity of local durian accession. The number of 41 local durian accession were taken from ex-situ collections in Hortimart Central Java. DNA accessions were isolated using modified CTAB method. DNA amplification used 4 PKBT-ISSR primers and the result was run through the agarose gel. Data were analyzed by NTSYS-pc program 2:02 version. Dendrogram created by the unweighted pair group with Arithmetical averages (UPGMA). DNA amplification produced 59 polymorphic loci and 7 monomorphic loci, and band size variation between 250 bp to 1500 bp. Accessions separated into two large groups at the coefficient 0.69, and the highest similarity coefficient is 0.95. Durian diversity in Central Java is included high level. Dendrogram showed there were only 4 pairs accessions which have a very high similarity. The couple of accessions are likely come from crossing a common ancestor. Then, the accessions spread and flourished in different areas, thus showing the difference in morphological characters but the molecular similarities are very high. The greater number of primers were used the more characters were observed, so it is possible the diversity level will increase and more accurate. In conclusion, the accessions analyzed are different accession.

Keywords: Durian; genetic diversity; ISSR

1. Introduction

Local varieties of durian in Indonesia amounted to very much that it is difficult to identify. This difficulty is caused by a lack of information about the characteristics of each variety so that the result is often a mistake. The most commonly trait used as a differentiator is the morphological of fruit. In addition the identity can be seen from the morphological of other organs, such as leaves, branches, and interest (Uji 2005). The limitations of morphological markers encourage the development of other marker that uses a material which controls the characteristics of an individual, namely DNA molecular markers. In plants, DNA molecular markers have advantages, namely stable and can be detected in all plant tissues and is not influenced by the environment (Kumar et al. 2009).

Inter simple sequence repeat (ISSR) is widely used as a molecular markers to reveal the diversity of the plants. ISSR is multilocus marker which is randomly generated by amplification of polymerase chain reaction (PCR) with microsatellites primers. The ISSR advantages are technically faster, less expensive, need smaal amout of DNA concentration as a tempate, and

capable of detecting genetic polymorphism without identify the genomic information (Kumar *et al*. 2009). ISSR molecular markers have been used to create a fingerprint of *Garcinia mangostana* germplasm (Widiastuti *et al*. 2013), the density of kinship cultivar identification of tangerine (Yulianti *et al*. 2010), and the identification of the genetic diversity of local durian Thailand (Vanijajiva 2011).

Hortimart Agro Centre is a garden collection located in District of Bawen, Semarang Regency, Central Java Province, Indonesia. The number of local durian ex-situ collection in Hortimart totaly of 110 accessions and until present the identity of each varieties is not clear because of the presence of sinonima not be divulged. Molecular markers can reveal the characteristics and identity of local durian accessions. All of the durian accessions were only named according to the name of puppet characters, examples Janoko, Suryo, and Bima. Molecular characterization using ISSR marker needed to reveal the genetic diversity because it can distinguish between individuals within a same species. The results can be used to determine the identity and provide the quality seeds information, so it will support a targeted multiplication program.

2. Materials and Methods

2.1. Materials and Prosedures

Subjects totally 41 accessions were taken from ex-situ collections in Hortimart Agro Centre, District Bawen, Semarang Regency, Central Java Province, Indonesia (Table 1).

Table 1. Name of durian accession ex-situ collections Hortimart Agro Centre

No	Accession	No	Accession	No	Accession
1	Jagal Bilowo	21	Dewi Sinto	41	Monthong
2	Ajimah	22	Pancatnyon		
3	Pancasona	23	Petruk		
4	Ponconoko	24	Ontorejo		
5	Suryo	25	Trijoto		
6	Pasopati	26	Semar		
7	Pendowo	27	Gareng		
8	Ontoseno	28	Mahesosuro		
9	Surtikanti	29	Ngalengko		
10	Bima	30	Abiyoso		
11	Yomodipati	31	Betorokolo		
12	Mustiko	32	GondomonL		
13	Banowati	33	embusuro		
14	Cokro	34	Rahwono		
15	Sugriwo	35	Arjuno		
16	Bismo	36	Jangkarbum		
17	Tirtonoto	37	Duryudono		
18	Romowijoyo	38	Anjani		
19	Ngastino	39	Kolosrenggi		
20	Janoko	40	Noroyono		

2.2. Extraction of total genomic DNA

DNA isolation procedures was modified and using the CTAB method (Vanijajiva 2011). They are consisted of four main stages, namely sampling leaves and extraction, purification, precipitation of DNA, and the DNA quality test.

Durian leaves crushed and added the extraction buffer consisting of 2% CTAB; 0.02 M EDTA pH 8.0; 0.1 M Tris-HCl pH 8.0; 1.4 M NaCl; β-mercaptoetanol 0.3 %. Then, the solution was incubated in a water bath at temperature of 60 °C for 30 minutes. Once removed then added a solution of phenol:chloroform:isoamyl-alcohol (PCI). PCI solution is used to separate proteins

that have been relegated from the buffer solution containing DNA. The solution added PCI then inverted until its bottom layer colour was changes. The solution was then centrifuged at 10,000 rpm for 15 minutes. Centrifugation causes the formation of three layers, the uppermost layer is the supernatant, the middle layer is the cell debris and the lower layer is chloroform. The solution in the uppermost layer was transferred to a new microtube. Supernatant was then added 0.6 x volume of 100 % cold isopropanol. The solution was inverted until the DNA precipitate. The next stage is centrifugation in 4000 rpm for 5 minutes. Superatan formed was then removed to the remaining pellets at the end of the microtube. DNA pellet was washed with 70% cold ethanol and then centrifuged at 4000 rpm for 5 minutes. Washing with cold ethanol did as much as 3 times. The supernatant was discarded and then the DNA pellets were dried at room temperature for one night. DNA pellets were dried and then reconstituted with TE buffer. The DNA isolation result seen in 0.8 % agarose gel.

2.3. Analysis of PCR-ISSR

Amplification is done on peqSTAR 2X PCR thermocycler machine. Primers used are ISSR primer of Pusat Kajian Buah Tropika (PKBT) (Table 2).

Table 2. Name and base sequens of PKBT-ISSR primer

No.	Primer name	Sequens	Annealing Temperature (ºC)
1	PKBT-2	(AC)8TT	53
2	PKBT-3	(AG)8T	53
3	PKBT-8	(GA)9C	54
4	PKBT-9	(GA)9T	54

PCR-ISSR compositions include DNA template, primer ISSR, the PCR master mix Promega go green tag and nuclease free water. Dilution is done in order to obtain the DNA concentration 10-50 ng/ml. Stages include predenaturation PCR, denaturation, annealing, extension, and final extension (Table 3).

Table 3. Steps and temperature on PCR-ISSR process

No.	Steps	Temperature	Time
1	*Predenaturation*	94ºC	4 menit
2	*Denaturation*	94ºC	30 detik
3	*Annealing*	36ºC-	30 detik
4	*Extention*	53/54ºC	1 menit
5	*Final extention*	72ºC	5 menit
7	Jumlah siklus	72ºC	35

Amplification products were separated on 1.2 % agarose gel. The size of each DNA band concluded with 50 bp DNA Ladder.

2.4. Data Analysis

The tape that appears is a particular allele. Genetic diversity was analyzed through dendrogram formed. The identity of each accession can be analyzed based on the genetic similarity coefficients were calculated using Dice coefficient (Nei and lei 1979). Genetic diversity matrix is then processed using similarity program for qualitative (SIMQUAL) DICE coefficient data in packets NTSYS-pc version 2:02 (Rohlf 1998). The analysis results formed a dendrogram based on unweighted pair group with arithmetical averages (UPGMA).

3. Results and Discussion

3.1. ISSR band profile

The amplification results using 4 primer of PKBT generate polymorphic band ranged from 250-1500 bp (Table 4).

Table 4. Band size variation of PKBT-ISSR primer 2, PKBT-ISSR primer 3, PKBT-ISSR primer 8, and PKBT-ISSR primer 9

No.	Primer	Band size (bp)
1	PKBT 2	330, 375, 400, 430, 450, 500, 575, 600, 625, 675, 700, 800, 875, 1000, 1250
2	PKBT 3	250, 300, 330, 375, 400, 450, 475, 500, 515, 575, 600, 625, 650, 675, 700, 750, 800, 875, 900, 1000, 1030, 1250
3	PKBT 8	313, 376, 400, 439, 500, 575, 600, 625, 700, 750, 800, 900, 1000, 1250
4	PKBT 9	250, 300, 330, 375, 400, 500, 600, 700, 750, 800, 875, 900, 1000, 1250, 1500

The DNA band profile showed in the following figures: PKBT 2 (Figure .1), PKBT 3 (Figure.2), PKBT 8 (Figure.3) and PKBT 9 (Figure.4).

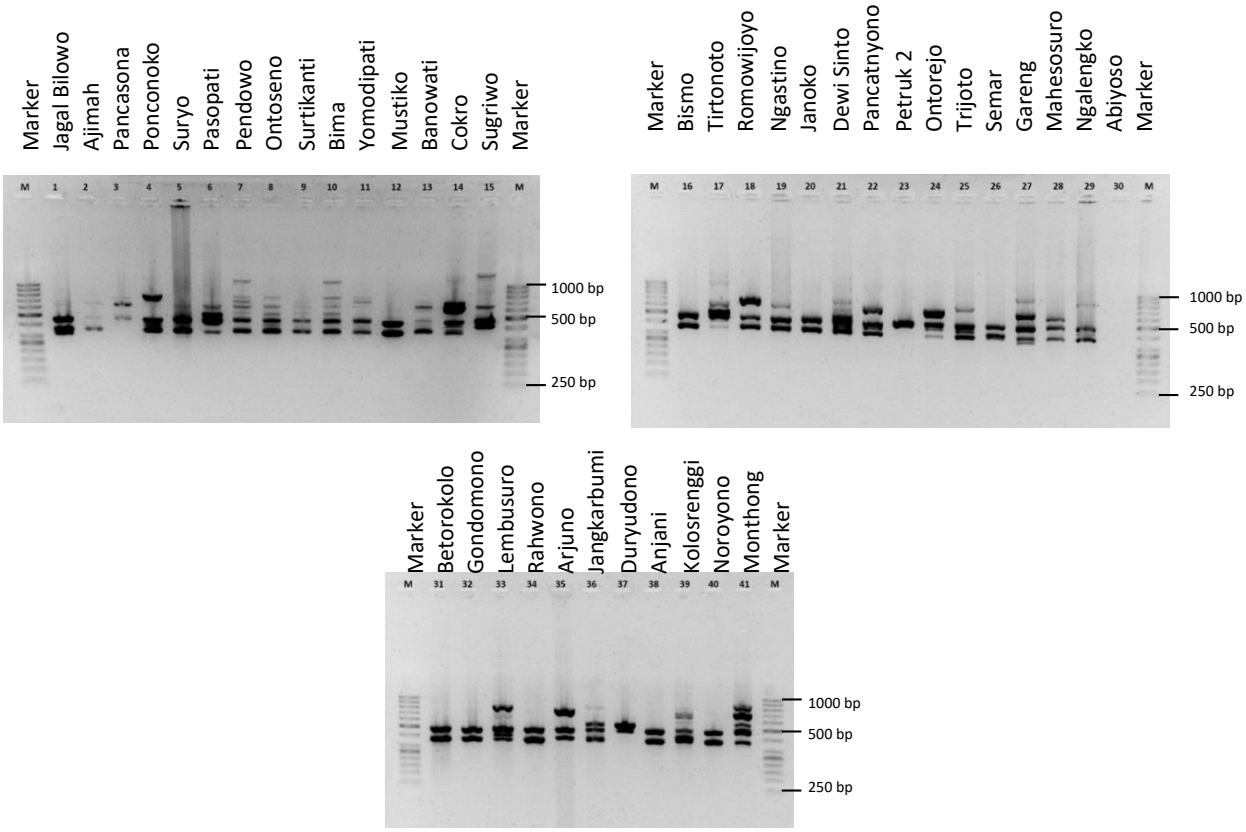

Figure 1. Profile DNA primer PKBT 2

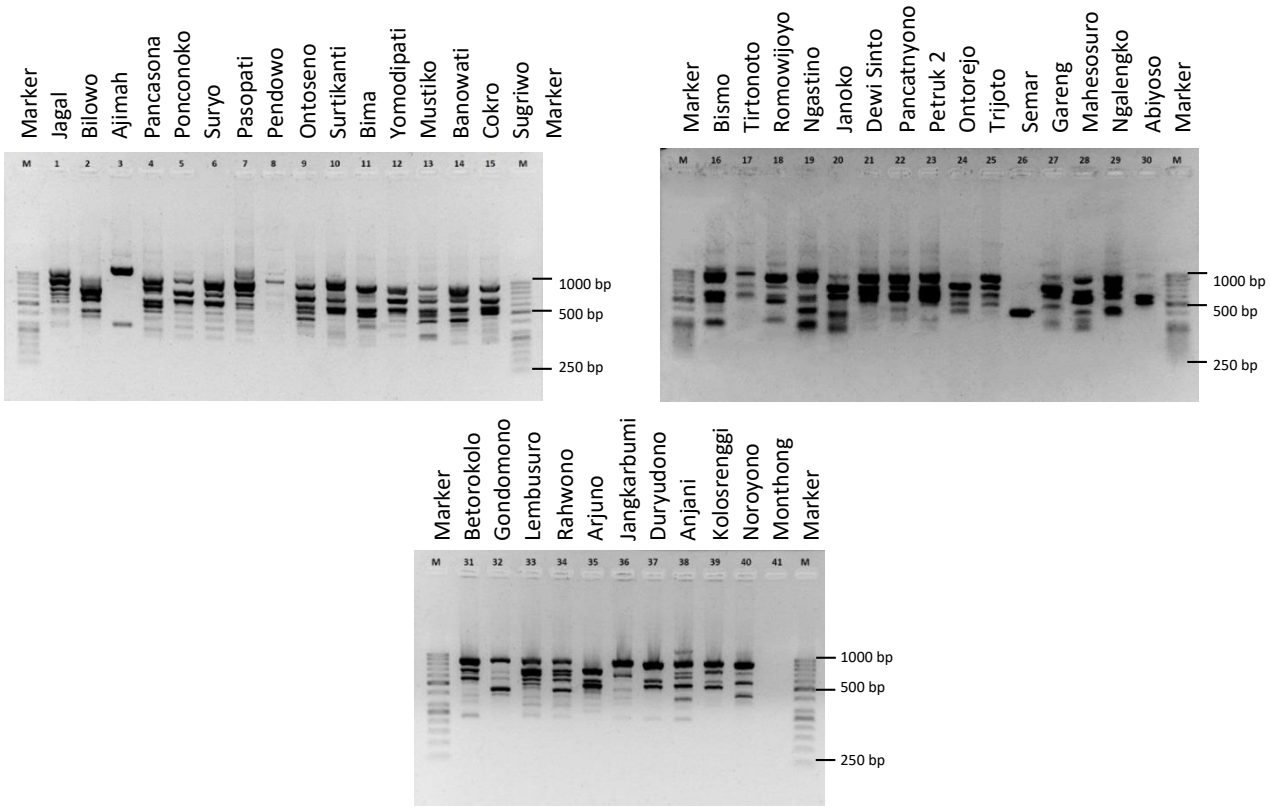

Figure 2. Profile DNA primer PKBT 3

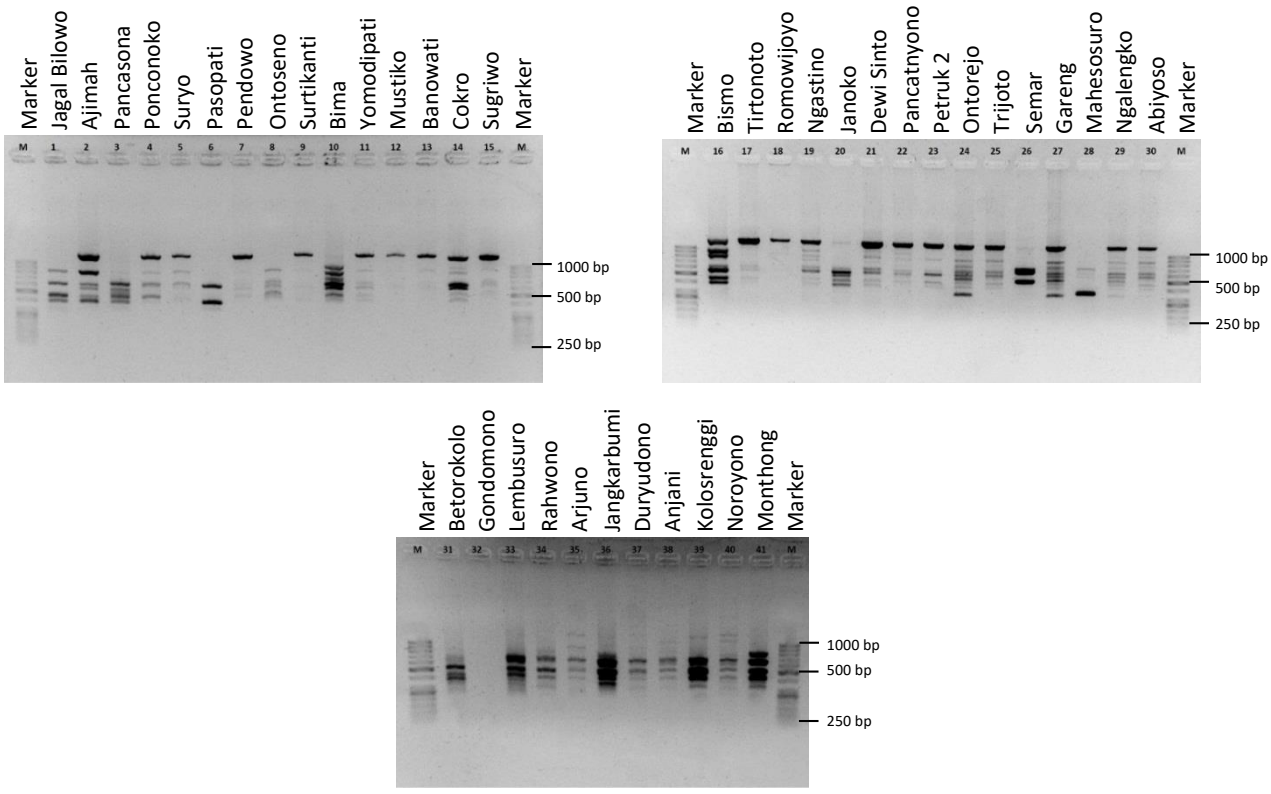

Figure 3. Profile DNA primer PKBT 8

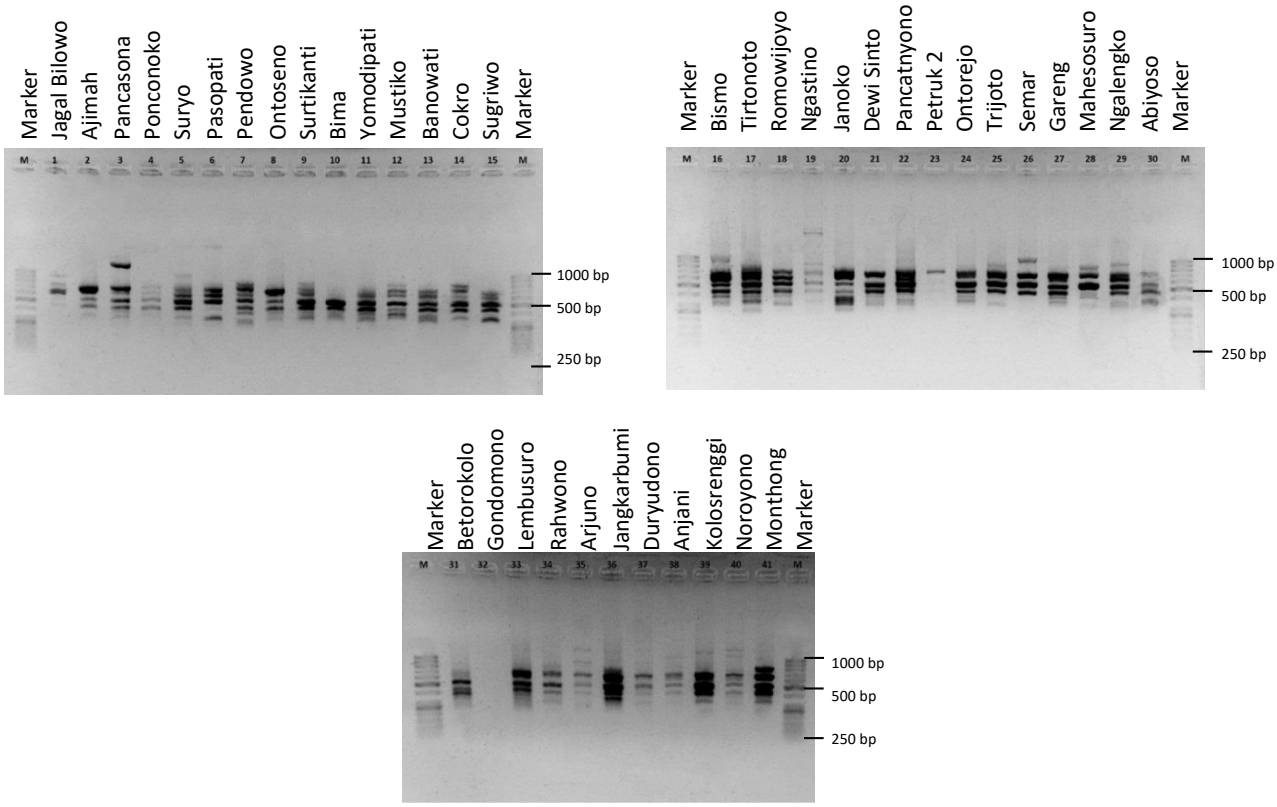

Figure 4. Profile DNA primer PKBT 9

The effectiveness of a marker assessed from the number of polymorphic band produced. The results of 41 samples of DNA amplification produces 729 band of DNA with 66 loci. Polymorphic band as much as 60 loci with 339 band or 73,84 % and monomorphic band as much as 6 loci with 178 band or 9.09% (Table 4).

Table 5. 4 ISSR primer amplification product in the 41 accession durian

Primer	Jumlah pita	Pita poli	Pita mono	Persentase polimorfisme (%)
PKBT 2	15	13	2	86,67
PKBT 3	22	22	0	100
PKBT 8	14	12	2	85,71
PKBT 9	15	12	3	80
Total	66	59 (89,39%)	7 (10,6%)	-

Analysis of local durian grouping relationship is presented in dendrogram (Figure 5).

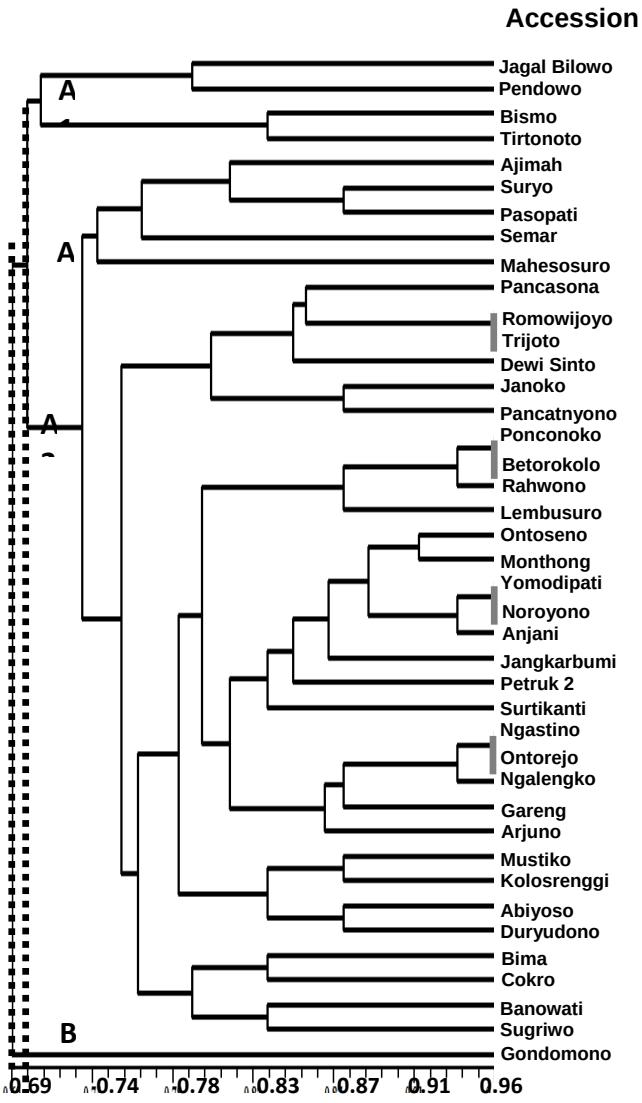

Figure 5. Dendrogram of 41 local durian accessions based on DNA band profile. Description: A and B are groups, A1 and A2 are subgroups.

3.2. Analysis grouping between durian accessions

ISSR primer produced polymorphic band from 80 % to 100% with band size variation from 250 to 1500 bp. The identification of D. zibethinus varieties in Thailand using RAPD only able to produce 23-50 % of polymorphic band for each primer. This fact indicates that ISSR markers has higher reproducibility than RAPD (Vanijajiva 2011). The value of polymorphic loci is over than 50 % indicates that ISSR marker is very informative and reliable markers. Reference (Botstein et al. 1980) shows the category of polymorphic classification value devided into three groups, more than 50 % is very informative, more or equal to 25 % and less than 50 % is moderate informative, and less than 25 % as less informative. The polymorphic value depends on the diversity of accessions tested.

ISSR marker has more profitable advantages than RAPD, namely able to produce the higher number of polymorphic band. ISSR marker is more informative and has been widely used to analyze the diversity within the same species and produce polymorphic band (Mansyah *et al*. 2010). The intensity of DNA amplification is heavily influenced by the distribution of primer

annealing site on a DNA template. The intensity of the band formation occurred due to the competition where the primary section attaches to single strand of DNA template. The existence of such competition caused many fragments are amplified at one site but on other sites are little bit. The amplification process can occur at several places, but only a few that can be detected as bands after amplification. The band pattern of specific DNA can be used to fingerprinting to distinguish a plant accessions (Kumar *et al*. 2009). The specific DNA band are useful for the identification of accession. The specific band is a specific marker or as a differentiator accession. This is useful as a solution for the morphological identification that require a long period in intensive observation. In addition, DNA fingerprinting has advantages, namely require the shorter time of identification, saving funds and manpower (Zulfahmi 2010).

The dendrogram formed shows the diversity of the local durian in Central Java is high level (Figure 5). All accessions studied only found four pairs which have a very high similarity. The couple accessions are (1) Romowijoyo-Trijoto, (2) Ponconoko-Betorokolo, (3) Yomodipati-Noroyono, and (4) Ngastino-Ontorejo, with similarity of 0.95. The couple of accessions are likely come from crossing a common ancestor. Accessions spread and cultivated in different region thus showing the difference of morphological characters but the molecular similarities are very high (Syahruddin 2012). Accession durian separated into two major groups in the coefficient of 0.69, which is A group and B group. The B Group consists only of one accession, namely durian Gondomono. PKBT-ISSR primer 8 separates Gondomono accession by specific band of 750 bp, and put it in B group. A group is divided into two sub-groups in the dendrogram coefficient of 0.70, namely: A1 sub group consisting the accession of Jagal Bilowo, Pendowo, Bismo, and Tirtonoto. A2 sub group consisting the accession of Ajimah, Suryo, Pasopati, Semar, Mahesosuro, Pancasona, Romowijoyo, Trijoto, Dewi Sinto, Janoko, Pancatnyono, Ponconoko, Betorokolo, Rahwono, Lembusuro, Ontoseno, Monthong, Yomodipati, Noroyono, Anjani, Jangkarbumi, Petruk 2, Surtikanti, Ngastino, Ontorejo, Ngalengko, Gareng, Arjuno, Mustiko, Kolosrenggi, Abiyoso, Duryudono, Bima, Clark, Banowati, and Sugriwo. The accession couples which have very high similarity contained in A2 subgroup.

Several factors affect the genetic variation of durian is pollination biology. Pollination biology is a factor that was important in the formation of durian diversity. The durian flowers are hermaphrodite cauliflorous and nature, so that self-pollination can still occur. Durian with the nature of self-compatible means that the pollen can be derived from the same flower or different flowers on the same tree and then pollinates the flower stigma of the same tree. Variations formed from self-compatible pollination will be a little as long as no pollination of pollen from another plant.

Morphological factors of stigma stalk position (stylus) is higher than the anther stalk (filamentum) as well as physiological factors that the difference of receptive stigma and dehiscense time of anthera is a factor that inhibits the occurrence of self-pollination. Durian flowers start blooming in the afternoon at about 4:00 p.m. to 4:45 p.m, but before blooming pistil usually already emerged from the flower buds first and receptive from 13.00 p.m until morning, while new anther has dehiscense at 19:30 p.m to 20:00 p.m (Bumrungsi *et al*. 2009), both of these factors lead to self-incompatible on durian. Lim and Luders (2009) show that the fruit formation by crossing manually only produce 31 % with better quality, contrastly the formation of the fruit by self-pollination is less than 10 % with the quality of the fruit is not good shape. Durian has three types of pollination, which are self-compatible, semi self-in compatible and self-incompatible. The pollination factors make some accession durian has a narrow diversity and some of them have a wide diversity.

ISSR molecular marker analysis based on a specific sequence contained in the DNA of plants relatively unaffected by the environment. Molecular markers can be used as characters or additional properties for the evaluation of the genetic diversity. Suggested grouping techniques preferably using molecular-based marker for relatively unaffected by the environment.

Molecular markers are based on specific genomic location and transmitted from one generation to the next in accordance with the laws of inheritance. Although molecular markers can not be likened to normal genes as molecular markers typically do not have biological effects and can be said as a constant marker in the genome, but can be used as a method to identify the specific genes on specific chromosomes as both are close to each other and tend to be inherited in every plant generation (Semagn *et al*. 2006).

The next factor is mutation in durian. The genomes of all organisms have many repetitive regions in the number and distribution. One of the important properties of this repeat sequence is have a tendency to higher mutation (Udupa and Baum 2001). The changes in DNA level is not necessarily followed by changes in the morphological level. This is understandable because a gene has a very long process to be expressed. Briefly involves two steps, namely the transfer of genetic information from DNA to RNA (transcription) and translation of genetic information contained in RNA into polypeptides (translation). There are so many factors that affect a gene expression. The values phenotype always contain the value of genotype, environmental deviation and interaction between genetic and environment. Morphological characters both quantitative and qualitative character are not only an expression of genetics, but also influenced by the environment. The difference of environmental circumstances provide the difference of morphological appearance (Wang *et al*. 2012).

Plant genetic diversity can be caused also by the activity of transposable elements. Certain codons are known as transposons have the ability to move around the genome, which can alter the expression of phenotype and cause somatic recombination. Somatic recombination of the different transposon effect of meiotic recombination as recombination occurs not only on homologous chromosomes, but also the entire genome. Somatic recombination lead some alleles can be transferred. Accessions that are experiencing these events will have more variation (Kalendar 2011). The insertion of transposable element followed by duplication sequence of base pairs in the host. The effect of insertion elements depending on their location. The insertion in non-coding region (the area without the genetic code) as the intron of the gene may hinder normal gene expression. Otherwise, the insertion in coding region can lead a frameshift mutation. Plant genomes are constantly changing both during cycle mitosis and meiosis. The mechanism of change include transpositions, translocation, amplification, and deletion. External environmental stress can induce a rapid mechanism of genomic changes. Genomic variation can be seen in a generation and can be transmitted to the next generation (Hoen and Bureau 2012).

The next factor of genetic diversity in plants depends on the distribution area. Plants which have geographically wide area of deployment will have a higher value than the diversity of plants with narrow deployment / endemic (Stuessy *et al*. 2012). Durian has a wide area of deployment and is very long-lived species, other than that the durian fruit is also favored by vertebrates. These characteristics make make durian has a high level of genetic diversity. The differences of environmental conditions cause plants to adapt in environmental conditions they lived. The plant adaptation can induce biochemical and physiological changes that affect the genetic diversity of these plants (Indriani *et al*. 2008).

This research is a preliminary study, so further analysis is required to identify each durian accession. The use of molecular markers ISSR is expected to have accurate results so that they can reveal genetic diversity of durian and support the development of plant breeding in the future.

4. Conclusion and Suggestion

Diversity of local durian in Central Java based molecular markers using 4 PKBT-ISSR primer is high level. The characterization results are presented in the a dendrogram. All of the accessions analyzed are different accession. The coefficient of 0.69 separated durian accession into two major groups, namely A groups and B group. A group separated into two subgroups at the coefficient of 0,71. Diversity will increase with the number of primers used. The greater number of primers were used the more characters were observed, so it is possible the diversity level will increase and more accurate. The analysis of 41 accessions of local durians is found only four couples of accession which have a very high similarity, namely Romowijoyo-Trijoto, Ponconoko-Betorokolo, Yomodipati-Noroyono, and Ngastino-Ontorejo with similarity coefficient of 0.95. Future studies are expected that the durian database consist of species name based on the analysis of PCR-ISSR can be arranged and used as a reference for classifying indentity of durian. A sequencing of PCR-ISSR products is important because the data has yet been found as supporting literature. DNA sequencing PCR-ISSR can be tracked to determine the percentage of similarity and homology using the BLAST program. The sama species which have a value of max score and the highest identity in the database.

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Vegetables

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Shallot Varieties Adaptation in Napu Highlands, Central Sulawesi

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Abstract

Napu highlands is one area in Central Sulawesi, which has a specific agro-ecosystem. This area has a wet climate'; rainfall> 2500 mm/year and has + 2,381 km2. Napu highlands altitude is 900-1200 m above sea level and is very suitable for development of vegetables, shallot (Allium cepa L). This study aims to determine adaptation of some shallot varieties in the highlands Napu. The Studies was conducted at the Wuasa village, North Lore Subdistrict, Poso in September-November 2015. There are three varieties tested; Sembrani, Bima, Maja and local Napu (control). The plant cultivation was used integrated crop management (ICM) principle. Area was used + 0.5 ha, and replicates 3 (three) units each variety. The plant growth and yield were collected and then averaged. The results of this study indicate Sembrani variety has highest yield and growth than three varieties, where productivity reached 30.8 t / ha, while local Napu lowest productivity (15.9 t / ha).

Keywords: Adaptation, shallot, highlands

1. Introduction

In Central Sulawesi, shallots grew and cultivated by farmers in the lowlands to the highlands. There is a change in the system of traditional subsistence farming to intensive cultivation and market-oriented. Unfortunately shallot production was not optimal and so much diversity in cultivation techniques. There are required cultivation techniques in specific agro-ecosystem.

The development of shallot in Central Sulawesi shows harvest area, production and productivity levels are typically increasing. Shallot production in 2014 amounted to 69 233 a quintal. Compare with 2013, production in 2014 increased by 18 204 quintal (35.67 percent). The increase in shallot production in 2014 resulted from the increased productivity of 13.61 quintals per hectare (34.85 percent), and the harvested area increased by 8 hectares or 0.61% (Central Sulawesi BPS 2015).

The average productivity of shallot in 2014 in Central Sulawesi was 5.26 ton/ ha, lower than the national average, which reached 10.22 ton/ ha (Directorate General of Horticulture, 2015). Low productivity is achieved due to less cultivation techniques applied by farmers; the use of suitable varieties of agro-ecosystem (lowland shallot varieties cultivated in the highlands). Planting shallot in highland cause crop plants has a longer lifespan, which can reach the age of 100 days. Bima that is shallot variety can widely adaptable.

Napu plateau is one area in Central Sulawesi, which has a specific agro-ecosystem. This area has a wet climate, and rainfall >2500mm / years with an area of $\pm$ 2,381 km^2. Napu plateau located at an altitude of 900-1200 m above sea level with a maximum temperature of 15^{o}C to 31^{o}C and minimum temperature as well as very suitable for development of vegetables,

including shallot (*Allium ascalonicum* L). The average productivity of shallot in this region is less than 5.0 t / ha (Syafruddin *et al.*, 1997).

Some shallot varieties cultivated in the lowlands relatively short age, varying between 55 to 70 days depending on the variety and the cropping season. Adaptability of shallot in Indonesia is quite extensive. The big difference in the life of shallot crop in the field to be harvested is a manifestation of the response of these plants to environmental influences and the most prominent is the agro-climatic conditions which occur between the lowlands to highlands, such as the state of the air temperature, evaporation, duration of solar radiation and solar radiation received each day, including differences in rainfall between the dry season and the rainy season in the lowlands and highlands. Climate significant differences between the lowlands and the highlands are temperature and sunlight, as well as differences in the sunlight between the rainy season and the dry season is very high occurs in lowland and highland.

IAARD through the Vegetable Research Institute in the last 30 years has been widely introduced shallot varieties, both for the lowlands, medium or high, but not yet widely known and used by farmers, so that the low production of local varieties are widely used. It is necessary for adaptation test varieties of shallot in the highlands so that the farmer can determine the types of adaptive varieties in its territory. The purpose of this study was to determine the adaptation of some varieties of shallot in the highlands Napu.

2. Materials and Methods

Studies conducted at Banyusari village, North Lore subdistric, Poso, Central Sulawesi in September to December 2015. The study used randomized block design with three (3) replicates. The treatments studied were shallot varieties; Sembrani, Bima, Maja and local Napu.

The plant cultivation was used integrated crop management (ICM) principle. After tilling perfect, planting beds were made with width 1.2 m along the plot of land. The soil was mixed with manure (4 t/ha), Biotriko and SP36 in 2 weeks before planting. Shallot spacing used 20 cm x 15 cm. Addition of Inorganic fertilizer was given 2 times; the dose were urea 200 kg / ha, 100 kg / ha and 300 kg NPK SP36. a week after planting, basic fertilizer; as half the dose of urea and NPK added to plant. Half the dose of fertilizer is given to plant after reaching one month old.

Weed control was done in two times before plant given additional fertilizer. Pest and disease control is carried out in integrated management. Pheromone exi traps were installed after shallot planting for 20 unit/ hectare. The biopesticide (Agonal) is also used in the study. Area was used $\pm$ 0.5 ha, and replicates 3 (three) units each variety. Plant growth and yield were collected and then averaged. Variable plant growth which were obeserved in study are plant height, number of leaf, the number of tubers per hill, tuber weight per clump, and productivity (t / ha). All observations were collceted untill harvest it.

3. Results and Discussion

Shallot Growth Performance

The result of the calculation of the average of the components of growth in four varieties of shallot plants showed the highest plant height is Sembrani (34.30 cm) and the lowest local Napu (25.07 cm), while the number of varieties achieved the highest leaf blade varieties Bima (27.23 sheet). The number of leaves correlates with the number of tubers. The more bulbs, the amount will be more leaves (Putrasamedja 2000; Kusmana et. Al 2009). Increasing the maximum number of leaves required by the plant, because the more the number of leaves, the higher photosynthesis activity to support plant growth and development. In detail, average growth component measurement results on the four varieties of shallot are presented in Table 1.

Table 1. Average plant height, leaf number and the number of tubers four shallot varieties at harvest time in the Highlands Napu 2015.

Treatment (Variety)	Plant Height (cm)	Number of leaves (sheet)
Sembrani	34.30	16.83
Maja	29.20	15.20
Bima	28.17	27.23
Lokal Napu	25.07	10.93

Yield Shallot Performance

The statistical analysis resulted from the average of the yield component. Although the number of tubers Sembrani only reach 5.17, but this varieties had the highest productivity of 30.80 t / ha with 87.14 g weights per clump. The Sembrani varieties was large tubers type, followed by Maja varieties, Bima and local Napu. The number of tubers are relate with the size of the bulb, where large-sized bulbs that have a fewer number of tubers (Kusmana et al. 2009). The detailed observation data (average yield) showed in Table 2.

Table 2. Average number of bulbs, tubers weight per clump and productivity of four varieties of shallot in the Highlands Napu 2015.

Treatment (Variety)	Tuber Quantity	Tubers weight per clump (g)	Productivity (t/ha)
Sembrani	5,17	87,14	30,80
Maja	7,60	51,30	27,10
Bima	11,00	52,43	23,80
Lokal	5,83	54,27	15,90

Pests and diseases of shallot during cultivation were lightweight category (small attacked). Caterpillar pest which was a *Spodoptera exigua* group attacked shallot in 2-4% percentage. The pest began attacking plant in 20 days old after planting. This was related with wet humidity (> 70%) around plant and average temperature reached above normal (30-32^{o}C). Shallot leaf which attacked by the pest was changed to silver/white. The low levels of pest attacked also related to application Pheromone exi traps in around planting area and biopesticide. Nurawan (2011) and Ocean (2010) reported that the use of pheromones exi effective in controlling

caterpillars shallots (Spodoptera exigua) by catched them 41,10- 83.83 per trap per week. Reducing pest populations will reduce percentage of attacks on plants.

The disease that attacked in shallot was spotting purple / trotol in lightweight category (<1%). The best of disease control was removed diseased plant before it spreading to other plants. The disease which caused by the fungus *Alternaria porii* was spread through tubers or splash of water in rain. Symptoms of attack marked the presence of concentric circles purple spots or white-gray leaves and on the edge of the yellow leaves and dry out the edges.

4. Conclusion

a. Adaptation of the four varieties of shallot showed the Sembrani varieties had highest results the 30.80 t / ha, followed by Maja (27.10 t / ha), Bima (23.80 t / ha) and the lowest local Napu (15.90 t /Ha).

b. Pests that attack during the shallot crop is caterpillar (Spodoptera exigua) in mild attack rate (2-4%) and purple spot disease / trotol (<1%).

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Collection and Characterization of Shallot Germplasm in Effort to Support National Food Security

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Abstract

Shallot is one of the important commodities in Indonesia because it's primary function as the main component of condiment almost in all dishes. It affect the demand for shallot will always exist and will rapidly increase belong to contribution of world population growth. So the increasing of shallot production should be carried out to maintain the stability of shallot suply. The increasing of shallot production can be maintained by the improvement of one or more characters of plants such as productivity, resistance to pests and diseases, and more else through plant breeding programs. The collection of genetic diversity through germplasm collection activity is the first step in the breeding programs. The collection of genetic diversity through germplasm collections could be obtained in several ways. Collection and identification process in order to shallot genetic diversity study was conducted by field exploration to several regions in Indonesia, the introduction and expansion of genetic diversity through radiation. The result of exploration and the introduction activities resulted on 79 genotypes collection, 35 genotypes among had been successfully characterized and testing the ability of flowering. The results showed that there was diversity within characters as well as of crown and bulbs characters and plants ability to the flowering time. Diversity was also demonstrated from the results of cluster analysis which divided the total of 35 genotypes into three major groups. In other hand, the result from genetic diversity expansion through mutations clustered a total of 55 genotypes into a group. Shallot germplasm collections were currently partially stored in the form of bulbs and some genotypes are stored in the form of botanical seeds/true shallot seed.

Keywords: Diversity, Genotype, Exploration, Introductions, True Shallot Seed

1. Introduction

Population growth and global climate changes is a challenge especially for the production of agricultural commodities including shallot. Shallot become one of the important commodities in Indonesia due it's primary function as a component of most entire cooking spice. It's presence as a major component in daily cooking make the shallot consumed almost every day by the people, especially in households. According to BPS data (2013) consumption of shallot in 2009 and 2010 at 2.52 kg / capita / year, in 2011 at 2.36 kg / capita / year, the year of 2012 amounted to 2.76 kg / capita / year, and the year of 2013 was 2.06 kg / capita /year. The data showed that consumption of shallot per capita/ year tend to be stable. So that the population growth will be positively correlated to the increasing of the national consumption of shallot. Shallot are sentisitif to the climate change. In addition to direct impact, climate change also affects indirectly through the development of pests and diseases. High rainfall is a

condition optimal conditions for the development of fungi that cause disease on shallot crop. Pests and diseases to be one of the limiting in shallot production.

Plant breeding program is one of the solution to the improvement of shallot in the future. Through plant breeding programs can be assembled plant that have the characteristics of high productivity to meet the challenges of increased production due to increased population. Plant breeding program is also able to assemble tolerant plants to environmental stress and resistance to pests and diseases to address the challenge of global climate change. The collection process is the first activity in the breeding program that aims to collect genetic diversity. Genetic diversity is important and major capital required in the breeding programs. Genetic diversity can be obtained through the exploration of several areas in the country, the alien plant introduction, hybridization, mutation, and genetic engineering. Therefore, activity of shallot germplasm collection is carried out in order to collect genetic diversity of shallots for shallot crop development in the future.

2. Methods

Shallots Germplasm collection

The collection process of shallots germplasm used several methods such as exploration into several regions in Indonesia including shallot production areas such as Brebes and Nganjuk; in collaboration with the National Council of Shallot, shallot farmers, and seed council; introduction shallots from many countries such as Thailand, Philippines, and Vietnam; as well as the expansion of genetic diversity by radiation. Germplasm were then planted with the purpose of propagation and conservation in the Garden Experiments II Tajur IPB Bogor, Bogor Experimental Farm Horse Sand and Sand Experimental Farm Sarongge Cipanas.

Characterization

Characterization started with the planting of the collection conducted in Experimental Farm of Pasir Kuda Bogor, Experimental Farm of Tajur II Bogor and Experimental Farm of Pasir Sarongge Cipanas. Planting methods used standart farming techniques for shallot cultivation. Shallot bulbs planted on land in lines with a spacing of 20 x 20 cm. Treatment for cultivation included irrigation, fertilizing and pest control. Characterization performed on both qualitative and quantitative characters for crown and bulb crops identification based on the individual observations guidebook (Kementrian Pertanian, 2013) and Calibration Book (Naktuinbow 2010). Morphological observation was focused on performation of canopy at 3 weeks old plants after planting (MST) or at the time of steady stage of vegatatif growth. Wherease, the observation of tubers morphological characters was in after harvest (8-9 MST).

Germplasm collections generated by radiation was still in the process of propagation and characterization visually through the observation of plant fitness especially tuber part. In every generation, individual plants that have the same fitness should be collected into a single slot genotype. Shallots planting was carried out in the Experimental field of Pasir Kuda-Bogor with plastic shade application to prevent rainwater. The cultivation techniques, plant spacing, and maintenance of the radiation plant collection was cariied out in the same way as a plant collection from exploration and introduction.

Flowering test

In addition to fitness observation of plant morphology, also done by observations on the plants ability to the flowering time. The research method for the plants ability to the purposed to the flowering induction was including the bulb vernalization at a temperature of 10 ° C for 21 days and the planting was carried out in the highlands position located in Experimental

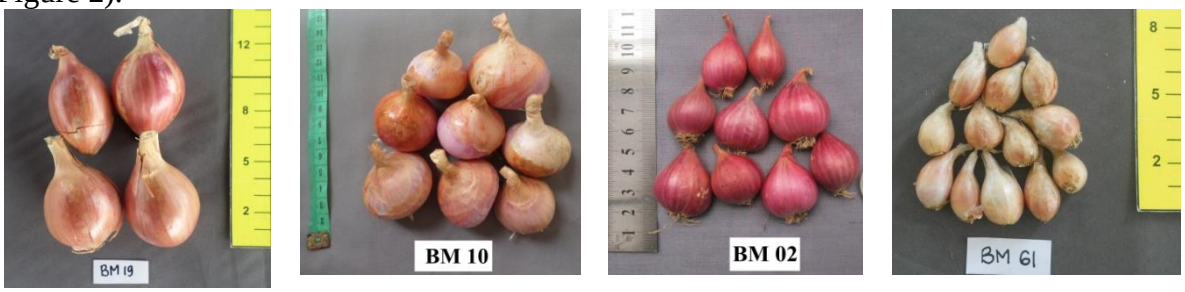
Garden of Pasir Sarongge. Planting was carried out by mounting with plastic shade applications with pacing of 20 x 20 cm. Plant maintenance was including irrigating, fertilizing, and control of plant intruder organism. Observations was made by counted the plants ability to produce flowers.

3. Results and Discussion

Results of exploration activities and the introduction of shallot germplasm managed to collect in total 79 genotypes and among a total of 35 genotypes have been successfully characterized the performance of its morphology. The results showed the existence of diversity in all the 35 genotypes. This diversity was apparent, especially on the characters contained bulbs. The diversity of tuber longitudinally shape were diverse into an oval shape-being, elliptical wide, round, rhombus, and elliptical cross-being (Figure 1).

Pic. 1 The diversity of tuber shape

Diversity on the tubers size was consisted of very large sizes, large, medium and small (Figure 2).

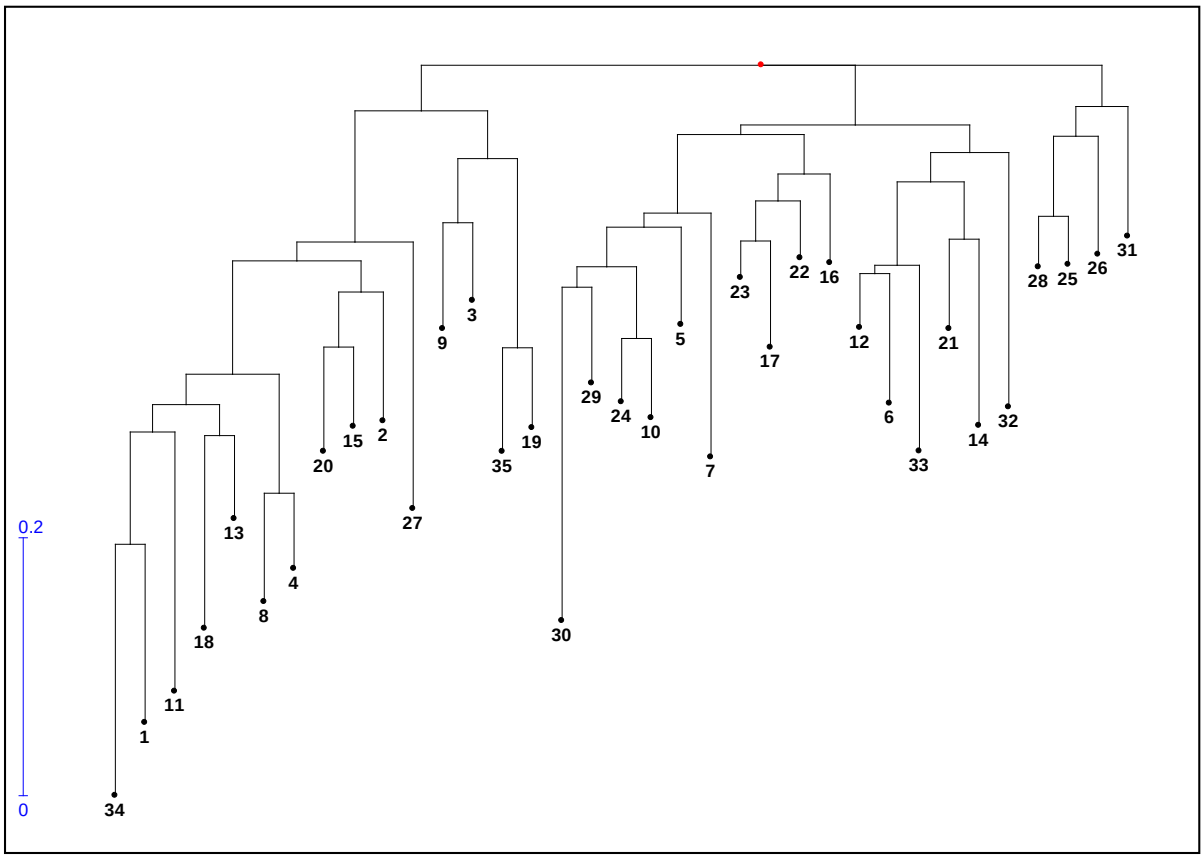


Pic. 2 Diversity on the tubers size

In other hand, the dominant color of dried tubers was red with a diverse color intensity, only one genotype that has color white bulbs.

Pic. 3 The dominant color of dried tubers

Diversity among 35 genotypes of collections that have been successfully characterized also evidenced by the results of cluster analysis using Darwin software version 6. The selected characters which performed in the similarities analysis has been evaluated in the previous study. Based on the results of cluster analysis, a total of 35 genotypes were divided into three major groups (Figure 4). Differences in group demonstrated the diversity that exists among genotypes. The genotypes that was in one group will likely have a close genetic distance and same performanced characters.



Note: 1 (BM 01), 2 (BM 02), 3 (BM 03), 5 (BM 05), 6 (BM 06), 7 (BM 07), 9(BM 09), 10 (BM 10), 11 (BM 12), 12 (BM 18), 13 (BM 19), 14 (BM 20), 15 (BM 24), 16 (), 17 (BM 25), 18 (BM 26), 19 (BM 28), 20 (BM 29), 21 (BM 47), 22 (BM 56), 23 (BM 57), 24 (BM 58), 25 (BM 59), 26 (BM 60), 27 (BM 63), 28 (BM 64), 29 (BM 65), 30 (BM 66), 31 (BM 67), 32 (BM 68), 33 (BM 72), 34 (BM 75), 34 (BM 22BM 76), 35 (BM 78)

Pic. 4 Cluster Analyis result based on 35 shallots collection genotype

The plant flowering ability test results also showed the diversity. Genotypes that were capable to flowering test among BM 01, BM 02, BM 03, BM 05, BM 06, BM 07, BM 10, BM 12, BM 18, BM 19, BM 20, BM 24, BM 25, 18 BM 26, BM 28 , BM 47, BM 57, BM 58, BM 59, BM 60, BM 63, BM 64, BM 65, BM 66, 31 BM 67, BM 68, BM 72, BM 75, BM 76 and BM 78. Wherease, the genotypes that did not generate interest on flowering test such as BM 09, BM 22B, BM 29, BM 36, BM 46 and BM 56. the ability of flowering in shallot crop is very important to understand because it is associated with the development of the seed in the form of botanical seed (true shallot seed) and for development of shallot through conventional plant breeding activities.

The result of the genetic diversity expansion through radiation was now successfully established diversity in total of 55 species. Genotypes which generted from radiation has not

been yet characterized and still on M4 generation progress and there was still diversity found in a single slot. Diversity was confirmed through visual observation mainly for bulb form characters. Shallot germplasm from exploration and the introduction were currently stored at the Center for Tropical Horticulture (PKHT) IPB as a collection for future study. The genotypes from exploration and the introduction collections was stored in the form of bulbs and botanical seeds for the botanical (Table 1). In other hand, the genotypes from radiation were all stored in the form of tubers.

Tabel 1 List of Shallots Collection

Genotype	Collection Form		Genotype	Collection Form		Genotype	Collection Form	
	Tuber	Seed		Tuber	Seed		Tuber	Seed
BM 01	√	-	BM 35	√	√	BM 60	√	-
BM 02	√	√	BM 36	√	-	BM 61	√	-
BM 03	√	√	BM 40	√	-	BM 62	√	-
BM 05	√	√	BM 41	√	√	BM 63	√	-
BM 06	√	-	BM 42	√	-	BM 64	√	-
BM 07	√	-	BM 43	√	-	BM 65	√	-
BM 08	√	-	BM 44	√	√	BM 66	√	-
BM 09	√	-	BM 45	√	-	BM 67	√	-
BM 10	√	-	BM 46	√	-	BM 68	√	-
BM 12	√	√	BM 47	√	-	BM 69	√	-
BM 14	√	-	BM 49	√	-	BM 70	√	-
BM 15	√	√	BM 50	√	-	BM 71	√	-
BM 16	√	-	BM 51	√	-	BM 72	√	-
BM 18	√	-	BM 52	√	-	BM 73	√	-
BM 19	√	√	BM 53	√	-	BM 74	√	-
BM 20	√	-	BM 54	√	-	BM 75	√	-
BM 21	√	√	BM 55	√	-	BM 76	√	-
BM 24	√	-	BM 56	√	-	BM 77	√	-
BM 25	√	√	BM 57	√	-	BM 78	√	-
BM 26	√	-	BM 58	√	-	BM 79	√	-
BM 29	√	-	BM 59	√	-			

4. Conclusion

Exploration, introduction and genetic diversity expansion activities through radiation managed to collect in total 134 genotypes of shallot. Based on the genotypes that have been successfully characterized, the genetic diversity between populations showed from shallot collection. Information flowering plants ability also successfully obtained from some genotypes. A total shallot germplasm has been collected, the diversity information was available and the flowering plants ability was characterized to be initial knowledge for the future development of shallot plants and cultivation.

References

Kementerian Pertanian. 2013. Guidelines for the conduct of test for distinctness, uniformity and stability [Onion (*Allium cepa* O. Fedtsch)]. Jakarta (ID): Kementerian Pertanian

Naktuinbouw. 2010. Calibration book *Allium cepa* (Cepa Group), *Allium cepa* (Aggregatum Group) and *Allium oschaninii* O; Fedtsch. and hybrids beetween the.

Optimum Fertilizer of Shallot on Andisol and Latosol Soils

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Abstract

Fertilization significantly increase growth and crop production, but is strongly influenced by the type of soil and crop varieties. This study aims to determine the optimum dosage of NPK fertilizer on growth and yield of shallot varietas Pancasona in two different soil types. Experiments carried on in the screen house using Split-plot design with three replications. The main plot (A): The type of soil, consisting of: a1 = Andisol-Lembang, and a2 = Latosol Red Yellow-Subang, while subplots (b): The dose of NPK fertilizer, comprising: b0 = 0, b1 = 500, b2 = 1000, 1500 = b3, b4 = 2000, and b5 = 2500 kg / ha of NPK (15-15-15) Phonska. The results showed that there was no interaction between the soil type and fertilization on growth and yield components. The type of soil and fertilizer single dose effect on plant growth (height and number of tillers) of shallot at age 49 HST. The optimum dose of fertilizer for shallot on Andisol is 1,055 kg NPK / ha with the results of the dry weight of 20.61 g /plant, while the optimum dose of fertilizer for onions in the ground latosol is 1,195 kg NPK / ha with a dry weight of 14.00 g /plant.

Keywords: NPK fertilizer, optimum dose, shallot

1. Introduction

The main characteristics of shallot cultivation in farmer's level is carried out in the paddy field cultivation, seedbed system with trench deep (> 50 cm), intensive crop management in terms of the use of NPK fertilizer and pesticides, but rarely using organic fertilizers. While the shallot seeds supplied (self-supply) - farmers with selecting and storing the tubers were excluded from the harvest as seeds in the next planting season (Crissman & Uquillas 1989). The existence of a sustainable farming system is implicitly a reflection of the reliability of the system of innovation in production to meet consumption needs and the needs of shallot seeds for farmers.

Nevertheless, the existence of new varieties and development of shallot to the area of production areas would require an adjustment of cultivation according to crop needs. Fertilizer for crop input requirements will depend on the level soil fertility, soil properties and characteristics, as well as other supporting environment crate climatic conditions. The effectiveness of nitrogen fertilization in addition to depending on the nature of the type of fertilizer used, the dose and the characteristics of the soil, also the ability of plants in their utilization. The effectiveness of fertilization in the cultivation of shallot associated with land application processing methods, application of chemical fertilizer-intensive in terms of dose and frequency of administration of fertilizer (Urea, ZA, SP-36 and KCl), and the irrigation system through watering plants. How Urea fertilizer or ZA is sprinkled on the surface of the soil / plant followed by watering, the water-soluble fertilizer, then partly into the ground and most of leaching and evaporates because it is usually held on the eve of the day.

One problem-solving efforts fertilization approached through research sources and dose of fertilizer, and how the application of effective appropriate crop management systems in the field. In line with the above description, the research activities directed to study shallot response to the dose of NPK fertilizer and soil types.

Survey results showed that the variation of the high dose of fertilizer N in the farmer's level of about 400-600 kg/ ha with the application system spread on the surface of the soil per plant followed by watering during the hot days will give an indication of inefficient use of fertilizers. On the other hand, the onion farmers in Brebes and Cirebon are not familiar with the use of organic fertilizer, which is necessary to evaluate the extent to which the availability of organic C helps increase the effectiveness of fertilization.

Improvement of soil fertility is the most practical is the addition of fertilizer to the ground. However, in addition to balancing soil fertility is also worth noting that fertilizers applied can be used by plants effectively and efficiently. Neither way is mainly nitrogen fertilizer applications to minimize the occurrence of changes in soil chemistry resulting GHG emissions either in the form of CO2 and N2O. Associated with it other than the way applications and application time from a variety of sources N needs to be improved, also the possibility of granting another ameliorant materials including functional microorganisms need to be assessed and evaluated its effect on the growth aspect and shallot, as well as their impact on the emission of CO2 and N2O.

2. Materials and Methods

Experiments in screen house performed for evaluation of response shallot growth and yield to the treatment NPK fertilizers and soil types, using a Split-Plot design with three replications.

a. The main plot, the type of soil (A):
   a1 = Andisol Soil - Lembang soil
   a2 = Red-yellow Latosol Soil - Subang

b. Subplot, the dose of NPK fertilizer (B):
   b0 = 0 kg / ha of NPK (15-15-15) Phonska
   b1 = 500 kg / ha of NPK (15-15-15) Phonska
   b2 = 1000 kg / ha of NPK (15-15-15) Phonska
   b3 = 1500 kg / ha of NPK (15-15-15) Phonska
   b4 = 2000 kg / ha of NPK (15-15-15) Phonska
   b5 = 2500 kg / ha of NPK (15-15-15) Phonska.

Shallot variety used is Pancasona. The type of soil used in this study is a red-yellow Latosol soil from Subang and Andisol soil from Lembang. Compound fertilizer treatment was applied to 2 x the ages of 10 and 30 days after planting. The size of the pot (2) kg soil with one plant per pot, plant maintenance carried out intensively for irrigation and pest control plant diseases.

3. Results and Discussion

Correlation

Here is a correlation between the results of soil analysis with variable observations on Andisol and Latosol Soils (Tables 1 and 2).

Table 1. Correlation between the results of soil analysis with variable observations on the Andisol Soil

	Dose of Fertilizer	pH	C (%)	N (%)	C/N	P_2O_5	K	Ca	Mg	PH 1	PH 2	PH3	TN1	TN2	TN3	FW	DW	WC
Dose of Fertilizer																		
pH	0,17																	
C (%)	0,79	0,65																
N (%)	0,66	0,70	0,79															
C/N	0,21	-0,51	-0,02	-0,53														
P_2O_5	-0,07	**0,82**	0,23	0,61	-0,79													
K	0,24	**0,88**	0,51	0,69	-0,54	**0,90**												
Ca	0,29	**0,93**	0,71	**0,86**	-0,62	0,76	0,76											
Mg	0,19	**0,99**	0,65	0,69	-0,50	**0,83**	**0,92**	**0,90**										
PH1	0,44	0,41	0,41	0,34	0,26	0,33	0,54	0,27	0,43									
PH2	0,27	-0,39	-0,26	-0,07	0,12	-0,04	0,08	-0,47	-0,31	0,25								
PH3	-0,14	-0,23	0,04	-0,19	0,19	-0,54	-0,65	-0,03	-0,31	-0,56	-0,76							
TN1	0,27	0,20	0,57	0,28	0,00	-0,25	-0,18	0,37	0,15	-0,42	-0,70	0,78						
TN2	0,53	0,37	**0,82**	0,41	0,20	-0,19	0,03	0,46	0,34	0,01	-0,60	0,56	**0,90**					
TN3	0,74	0,31	**0,88**	0,46	0,37	-0,23	0,06	0,39	0,28	0,27	-0,38	0,39	0,72	**0,94**				
FW	-0,10	-0,64	-0,26	-0,28	0,13	-0,68	**-0,84**	-0,37	-0,70	-0,73	-0,29	**0,81**	0,50	0,19	0,08			
DW	0,09	-0,50	-0,09	-0,01	0,02	-0,52	-0,70	-0,16	-0,57	-0,54	-0,28	0,75	0,48	0,22	0,17	**0,94**		
WC	-0,16	-0,47	-0,31	-0,67	0,54	-0,53	-0,33	-0,70	-0,40	-0,25	0,37	-0,12	-0,11	-0,11	-0,13	-0,02	-0,32	

Table 2. Correlation analysis results with variable ground observations on the Red Yellow Latosol Soil

	Dose of Fertilizer	pH	C (%)	N (%)	C/N	P_2O_5	K	Ca	Mg	PH 1	PH 2	PH3	TN1	TN2	TN3	FW	DW	WC
Dose of Fertilizer																		
pH	0,24																	
C (%)	0,31	**0,91**																
N (%)	-0,30	0,53	0,40															
C/N	0,64	0,41	0,57	-0,51														
P_2O_5	-0,03	**0,91**	**0,83**	**0,82**	0,05													
K	0,25	**0,97**	**0,87**	0,37	0,53	**0,81**												
Ca	0,38	**0,85**	**0,83**	0,70	0,20	**0,88**	0,72											
Mg	0,16	**0,97**	**0,92**	0,69	0,27	**0,97**	**0,89**	**0,93**										
PH1	0,30	0,02	-0,28	0,20	-0,30	-0,01	-0,06	0,21	-0,01									
PH2	0,56	-0,37	-0,31	-0,33	0,04	-0,43	-0,34	-0,19	-0,39	0,10								
PH3	0,25	-0,67	-0,56	-0,44	-0,15	-0,66	-0,63	-0,51	-0,66	-0,09	0,92							
TN1	**0,95**	0,03	0,04	-0,30	0,41	-0,19	0,01	0,25	-0,03	0,53	0,61	0,34						
TN2	**0,93**	-0,03	0,08	-0,27	0,38	-0,19	-0,08	0,28	-0,04	0,41	0,62	0,38	**0,97**					
TN3	**0,93**	0,00	0,11	-0,24	0,39	-0,16	-0,06	0,31	0,00	0,41	0,59	0,35	**0,96**	**1,00**				
FW	0,55	-0,38	-0,33	-0,23	-0,08	-0,40	-0,40	-0,12	-0,38	0,21	**0,98**	**0,89**	0,64	0,67	0,64			
DW	0,41	-0,42	-0,19	-0,05	-0,16	-0,29	-0,53	0,02	-0,28	0,00	0,74	0,74	0,48	0,65	0,64	**0,81**		
WC	0,12	-0,03	-0,36	-0,05	-0,20	-0,13	0,06	-0,18	-0,17	0,47	0,47	0,35	0,23	0,02	-0,01	0,44	-0,13	

Soil Analysis

The results of soil analysis before and after the experiment can be seen in Table 3. Application of lime at 2 tons per hectare can improve soil pH both on the Latosol and Andisol Soils. Increased soil pH ranging 0.1 on Andisol Soil until 2.0 on Latosol Soil. Lime makes nutrients become more available to plants.

Table 3. Results of soil analysis before and after the experiment

Treatments	pH	C (%)	N (%)	C/N	P_2O_5 (ppm)	K (ppm)	Ca (cmol/kg)	Mg (cmol/kg)
Before Experiment (Andisol Soil)								
A1	5,5	5,66	0,62	9	1417,8	213,4	8,03	1,90
After Experiment (Andisol Soil)								
A1B0	5,6	6,27	0,65	10	763,8	1624,3	13,81	3,72
A1B1	6,5	6,90	0,73	9	1038,7	2508,4	20,23	6,32
A1B2	5,5	6,70	0,68	10	570,0	845,7	15,29	3,17
A1B3	5,8	6,67	0,73	9	900,4	2066,5	16,94	4,33
A1B4	5,8	6,86	0,72	10	779,7	1733,9	16,81	4,15
A1B5	6,2	7,27	0,73	10	813,1	2387,9	17,88	5,63
Before Experiment (Latosol Soil)								
A2	4,6	1.05	0,18	6	3,5	218,3	2,50	0,47
After Experiment (Latosol Soil)								
A2B0	6,6	2,90	0,29	10	1649,7	2692,0	13,55	5,20
A2B1	5,3	1,39	0,17	8	918,1	1270,7	8,23	1,76
A2B2	6,2	2,54	0,25	10	1423,9	2308,7	12,11	4,12
A2B3	6,0	2,66	0,26	10	1387,8	1820,3	13,76	4,15
A2B4	6,4	2,42	0,28	9	1482,8	2375,3	14,50	4,58
A2B5	6,4	2,92	0,16	18	1294,5	2624,2	12,66	4,22

Plant Growth

The type of soil and fertilizers in general does not affect the growth of plant height and number of tillers. At the age of 49 days after planting (dap), soil type real effect on plant height (Table 4), the plants on soil type Andisol provide plant height higher than plants in Latosol soil. However, there is no interaction between the soil type and dose of fertilizer on plant growth, both at the age of 15, 35 and 49 days after planting.

Table 4. Effect of soil type and fertilizer dossage on height of plant

Treatments	Height of Plant		
	15 dap	35 dap	49 dap
Main Plot:			
A1=Andisol	6.66 (ns)	11.96 (ns)	26.80 a
A2=Latosol	6.42	11.70	21.23 b
Sub Plot:			
b0 = 0 kg NPK/ha	6.50 (ns)	11.33 (ns)	23.32 (ns)
b1 = 500 kg NPK/ha	6.57	11.77	24.57
b2 = 1000 kg NPK/ha	6.24	12.03	25.73
b3 = 1500 kg NPK/ha	6.40	11.97	23.53
b4 = 2000 kg NPK/ha	6.93	11.97	23.60
b5 = 2500 kg NPK/ha	6.60	11.90	23.33
CV (%)	11.86	9.63	9.09

At the age of 49 days after planting, fertilizing effect on the number of tillers, while the soil factors had no effect on the number of bulbs (Table 5). NPK fertilizer dosage 666 kg per ha and 1333 kg NPK per ha significantly different from the control or 0 kg NPK per ha, while the other doses were not significantly different. Of the two tables, it can be seen that the influence of the soil type and dose of fertilizer on plant growth until age 49 dap which is the optimum growth of the onions. The type of soil affect plant height while fertilizers affect the bulb number of shallot.

Application of NPK can increase the number of tillers (Table 5). This is because the Nitrogen is involved in the formation of amino acids, proteins, nucleic acids, enzymes, nucleoprotein and alkaloid that is needed in plant growth, especially the growth of leaves, increase the green color of leaves as well as the establishment of branches or tillers (Nasreen et al., 2007, Abdissa et al 2011).

Table 5. Effect of soil type and fertilizer dossage on number of bulb

Treatments	Number of Bulb		
	15 dap	35 dap	49 dap
Main Plot:			
A1=Andisol	3.60 (ns)	3.83 (ns)	4.29 (ns)
A2=Latosol	3.64	3.93	3.93
Sub Plot:			
b0 = 0 kg NPK/ha	3.00	3.00	3.00 b
b1 = 500 kg NPK/ha	3.67	3.75	3.75 ab
b2 = 1000 kg NPK/ha	4.00	4.00	4.50 a
b3 = 1500 kg NPK/ha	3.27	4.25	4.00 ab
b4 = 2000 kg NPK/ha	3.67	4.25	4.75 a
b5 = 2500 kg NPK/ha	4.00	4.00	4.20 ab
CV (%)	18.80	9.64	9.64

Shallot Production

The percentage of plants harvested, fresh weight and dry weight per plant can be seen in Table 6. There is no real influence of soil type and fertilization to the percentage of harvested plants, but the soil type significantly affected fresh weight and dry weight per plant. Fresh weight and dry weight on Andisol Soil significantly higher than the fresh weight and dry weight in Latosol soil.

Tabel 6. Effect of soil type and fertilizer dossage on yield component of shallot

Treatments	Percentage of Harvested Plant	Fresh Weight per Plant (g)	Dry Weight per Plant (g)
Main Plot:			
A1=Andisol	93.33 (ns)	24.11 a	18.03 a
A2=Latosol	95.56	17.05 b	11.98 b
Sub Plot:			
b0 = 0 kg NPK/ha	90.00 (ns)	16.88 (ns)	12.87 (ns)
b1 = 500 kg NPK/ha	96.67	19.34	13.85
b2 = 1000 kg NPK/ha	96.67	22.83	17.07
b3 = 1500 kg NPK/ha	96.67	22.10	16.56
b4 = 2000 kg NPK/ha	96.67	22.50	15.32
b5 = 2500 kg NPK/ha	90.00	19.83	14.46
CV (%)	11.62	28.17	31.59

In different soil types, fresh weight of bulb per plant significantly different. Fresh weight and dry weight per plant on the Andisol Soil significantly higher than the Latosol Soil. This is in line with the results of research Ajayi et al (2006) in plants Yam (Dioscorea species) in which the different types of soil affect tuber formation. Similarly found in wheat plants that contain different nutrients affect the entire plant fresh weight (Azam & Lodi 2001).

Optimum dosages for fresh weight bulb yield per plant on Latosol and Andisol soils can be seen in Figure 1, where the influence of the response is a graph polynomials with optimum dosage of 980 kg NPK/ha for land Andisol with results 26.49 g/plant. While the optimum dose for Latosol soil is 1145 kg NPK/ha with the results of wet weight 19.90 g/plant.

Fertilization response graph of the dry weight of plants in two different soil types can be seen in Figure 2. In Andisol Soil, optimum dose for the dry weight is 1055 kg NPK/ha with a dry weight 20.61 g/plant. While on Latosol Soil optimum dose is 1195 kg NPK/ha with a dry weight 14.00 g/plant. Of the two figures, it can be seen that the optimum dose in Latosol soil is higher than on the Andisol soil, besides the fresh weight and dry weight were obtained in Latosol soil was lower than in Andisol soil. This proves that the land is poorer than Andisol soil. In addition to the dose of fertilizer should be noted also the time of application and how fertilization, because the time of application of fertilizer that may cause loss of nutrients so inefficient (Smiciklas & Moore, 2008), as well as how an application can affect crop yield (Moghaddam et al., 2007). Besides the addition of chemical elements, should also be carried improvement in the physical properties and biological soil.

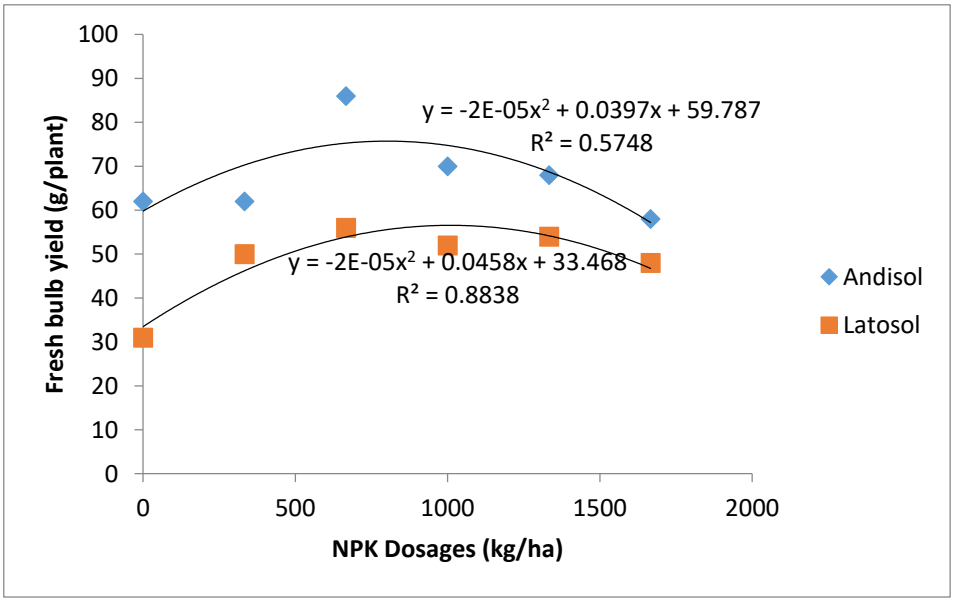

Figure 1. Graph response to the wet weight of fertilization on Andisol and Latosol Soils.

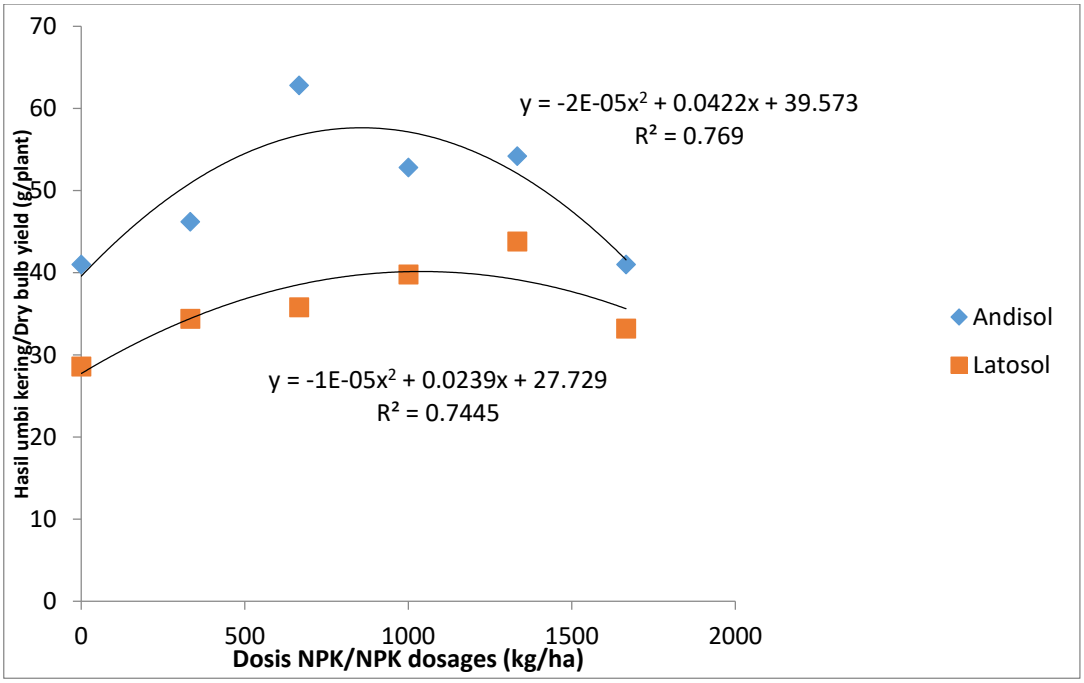

Figure 2. Graph response to the dry weight of fertilization on Andisol and Latosol Soils.

4. Conclusion

1. The type of soil and fertilizers affect plant growth (height and number of tillers) of shallot at age 49 dap. However, there is no interaction between the soil type and dose of fertilizer on plant growth.
2. Dose optimum fertilizer for shallot in Andisol soil is 1055 kg NPK/ha with the results of the dry weight of 20.61 g/plant.
3. Dose optimum fertilizer for shallot in Latosol soil is 1195 kg NPK/ha with the results of the dry weight of 14.00 g/plant.

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Effect of Organic Fertilization on The Growth and Yields of New Onion Varieties in Limited Land

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Abstract

Cultivation of plants in a limited area / yard has a low yield due to less intensive treatment. Recommended application of organic fertilizers in the onion that is 20-25 tonnes / ha was responded negatively by farmers, so need an alternative way to obtain efficient cultivation. The objective of research was determining the growth response and the results of several new varieties of onion with using commercial organic fertilizer in a limited area / yard. The research was conducted in the greenhouse Sidondo Experimental Garden in November 2015 - February 2016. Using a randomized complete block design (RAK) with several new varieties of onion (Pancasona, Mentes, Trisula and Pikatan), each of which was repeated 5 times. Growth media is soil mixed with organic fertilizer (Petroganik) with a ratio of 1: 2, and maintained on board tub size of 1 m x 0.60 m x 0.20 m. The results showed that the Pikatan varieties provide productivity gains of 9.87 tonnes / ha higher than other varieties but less than in the field that can obtained 15.89-23.3 tonnes / ha. Growth response (plant height and number of leaves) was not optimal when compared to plant in the field (57-70%) because it was lack of content of nitrogen and potassium.

Keywords: organic, onions, VUB, limited area

1. Introduction

Productivity onion in Central Sulawesi in the last two years was 3.37 t/ha and 4.20 t/ha, increased 24.76% (Agriculture Ministry, 2015). The low shallots productivity in Central Sulawesi due to application of recommended technology package on farmers remains low. In production area (Tomini, Tinombo and Sigi Biromaru), the cultivation was old way which used local variety and cultivated for many generations (seeds which used were taken from the previous harvest). The shallot productivity can be improved by introducing new high yielding varieties that have high yield potential and can be planted in the rainy season.

Shallots is a commodity that has a high risk and unstable prices (Setyono and Purwaningsih, 2006). The farming activities require large input costs (seed cost), the tendency of farmers use excessive pesticides to protect plants from pests and diseases, so that there is a residual-risk chemicals in garlic are harmful to human health. The onion which is a vegetable is often consumed by people as a spice in cooking. Karyadi (2008) reported that the effects of excessive pesticide use can damage the soil and surrounding environment; it can increase the content of lead (Pb) in soils of 43.071.6 mg/ha.

IAARD; Balitsa had released several varieties of onion which is yielding varieties and high productivity, which are Bima Brebes, Maja, Cipanas, Kramat 1, Kramat 2, Kuning, Sembrani, Katumi, Mentes, Pancasona, Pikatan, and Trisula. Red onion varieties; Pikatan, Pancasona, Trisula and Mentes are suitable seeded to be planted in the rainy and dry season. The cultivation technique was similar in both seasons. Adaptation test of varieties in specific area needed to

determine the suitability of the environment, the potential yield, tolerance against plant pests as well as the age and quality of the results. There are factors of improved varieties can be accepted and adopt by farmers.

The use of high yielding varieties can increase the production of onion plants. Bakhri et al., (1999) reported that the use of high yielding varieties of tubers Philippines can produce 10,350 kg / ha with Rp.10.634.00- income / ha higher than the local varieties but not coupled with the efficiency of farm inputs. The high level of use of pesticides in the cultivation of this demanding technological improvements that lead to organic products that are free of pesticides. This study aimed to determine the effect of organic fertilizers on growth and yield several varieties of onion.

The use of high yielding varieties can increase onion production. Bakhri et al., (1999) reported Philippine varieties can produce tubers of 10,350 kg / ha with Rp.10.634.00- / ha in revenue higher than the local varieties but not efficient on farm inputs. The high use of pesticides in the cultivation of these required changes in the application of technology; organic products free from pesticides. This study aimed to determine the effect of organic fertilizers on growth and yield several varieties of onion

2. Materials and Methods

This research was conducted in the greenhouse; Sidondo Experimental Park in November 2015 - February 2016. The design used was a randomized block design (RAK), a factor that was tested new varieties of shallot (Pancasona, Mentes, Trisula and Pikatan) that are repeated five times for each variety. Planting medium was used soil mixed organic fertilizer (Petroganik) with a ratio of 1: 2, then each Shallot plan on board tub size of 1 m x 0.60 m x 0.20 m. Spacing was applied 20 cm x 10 cm. Plants was watering every day according plant needs. Pest and disease control was done manually without pesticides; if plant attacked by the disease then removed it quickly to reduce contagion. Variables measured are plant growth and yield such as plant height, number of leaves, wet bulb weight per clump and productivity. After data received, then tthe data were analyzed using a randomized block design mathematical formula as follows:

$$Y_{ij} = u + t_i + b_j + E_{ij}$$

i = 1,2,….,6 and j = 1,2,….,x
Y_{ij} = Observations on the treatment of i and j (group)
u = Average all value
t_i = Effect of treatment of i
b_j = Effect of group of j
E_{ij} = Random effect of treatment i and j (group)

3. Results and Discussion

Growth Component

Plant growth showed height and number of leaves formed by using only organic fertilizers (Petroganik) without the addition of inorganic fertilizers generate normal plant growth and similar with genotype of each variety. Lee, (2010) reported that organic fertilizers provide a low impact on plant height, leaf number and the number of tubers formed compared to the use of chemical fertilizers. He also explained that organic fertilizers did not significantly affected in onion crops weight. Observations on the plant height showed that the varieties Pikatan

provide the highest value of 24.19 cm but not significantly different with the three other varieties (Pancasona, and Test the Trident). The high crop varieties Pikatan followed by the high number of leaves formed. The mean height and number of leaves on each treatment showed in Table 1.

Table 1. Average height and number of leaves on each shallot variety

Variety	Height (cm)	Avg. height (cm) in field, (Brebes)[1]	Avg. height (cm) in field, Tegal[1]	Avg. height (cm) in field, Nganjuk[1]	Number of leaves
Pancasona	23.43a	44.63	41.07	37.47	9.15a
Mentes	23.29a	40.27	33.30	32.80	10.78a
Trisula	21.69a	38.13	38.10	32.47	8.41a
Pikatan	24.19a	37.03	34.37	32.47	12.20a

Description: If the same letter in the same column are not significantly different at the level of 5% Test LSD
[1] Hidayat et al (2011) in rainy season

In Table 1 showed differently shallot condition (height) by planting in field and board tub. Hidayat et al (2011) reported average height of Pikatan, Trisula, Pancasona, Mentes varieties; 39 cm, 39,92 cm, 41,3 cm and 42,07cm. Growth response (plant height and number of leaves) was not optimal when compared to plant in the field (57-70%) because it was lack of content of nitrogen and potassium. Alternative reason which shallot height was not optimal was limited availability growth space and development tubers.

Yield and Productivity

Optimal plant growth has an impact on onion yield. The results showed that the Pikatan have heavy wet bulb per clump higher than the three other varieties. The mean weight of tuber per panicle and the productivity of each treatment showed in Table 2.

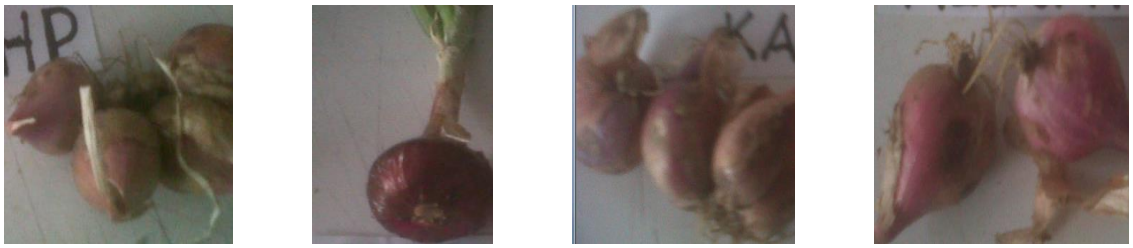
Tabel 2. Mean Tuber weight per panicle and the productivity of each treatment.

Variety	Wet weight of tuber /panicle (gr)	Productivity (t/ha)	Productivity in field[1] (t/ha)
Pancasona	10.47b	6,28b	10.67
Mentes	9.99b	5,99b	9.41
Trisula	10.20b	6,12b	9.34
Pikatan	16.45a	9,87a	8.01

Description: If the same letter in the same column are not significantly different at the level of 5% Test LSD
[1] Hidayat et al (2011) in rainy season

In Table 2 showed that application of organic fertilizers (Petroganik) without additional inorganic fertilizer able to produce high productivity in all varieties of onion. Productivity is highest on the Pikatan; 9.87 t / ha and significantly different to the three other varieties (Pancasona, Mentes and Trisula). Similar with Table 1, Table 2 also showed differently shallot productivity by planting in field and board tub. Hidayat et al (2011) reported average productivity of Pikatan, Trisula, Pancasona, Mentes varieties were 6.20-23.31 ton/ha, 6.50-23.21 ton/ha, 6.90-23.70 ton/ha, and 7.10-27.585 ton/ha.

Media, planting time and population density onions greatly affect the productivity of onion plants produced (Caruso et al., 2013). Furthermore, the color bulbs quality of each variety can be seen in Figure 1.



Mentes Trisula Pancasona Pikatan

Figure 1. Display color bulbs each treatment

Trisula had a bright red color, followed by Pikatan, Mentes and Pancasona. Pancasona had a large bulb sizes but the bulbs was single. Mentes had more tuber per clump than other varieties, but only small-sized tubers had produced. Rajiman, (2010) reported that the use of inorganic fertilizers only affects productivity but does not affect the quality of onion. The quality onion that is generated by using only organic fertilizers had met standard.

4. Conclusion

Pikatan variety had higher productivity compared to the three other varieties. Application of organic fertilizer (Petroganik) provided optimal growth and yield in Pikatan, Pancasona, Trisula amd Mentes. Further research in optimized onion productivity by mixed organic and inorganic fertilizer (suggestion).

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Interaction Between Varieties and Plastic Mulch on Shallot Growth in Dryland South Kalimantan

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Abstract

Demand for shallot is continuing to increase (by an estimated 5% per year), in line with the increase of population of Indonesian people, more over, shallot is one of some comodities that cause economic inflation In Indonesia. Potential dry land in South Kalimantan also large enough to develop shallot. The aim of this study was to determine the effect of the interaction between the use of black silver plastic mulch and varietties of shallot on the shallot growth in dry land South Kalimantan. The assessment was done on dry land in the Kunyit village Tanah Laut regency and Karangrejo village Banjarbaru Municipality in 2015 arranged in Randomized Block Design with 2 factors : first factor was 8 varieties, namely Bima Brebes, Sri Kayangan, Rubaru, Biru Lancor, Super Philip, Bauji, Crok kuning, Keta monca. The second factor was planted with plastic mulch and without plastic mulch.. The results show that there is interaction between plastic mulch and varieties. The highest production was got by Super Philip varieties are grown with plastic mulch is 26.4 t / h (Kunyit Village, Tanah Laut District) and 15.4 t / h (Karangrejo Village Banjarbaru Municipaly). Conclude that in dry land South Kalimantan planted shallot with plastic mulch had higher production than without mulch.

Key words : mulch, shallot varieties, dry land

1. Introduction

Shallot consumption of Indonesian people per capita per year to reach 4.56 kg or 0.38 kg/capita/month. Therefore the demand for shallot will continue to increase (by an estimated 5% per year), in line with the increase of population, the development of the processing industry, the development of export markets, and as a source of income and employment opportunities contribute high enough on the economic development area (Directorate General of Horticulture, 2008).

On the other hand, South Kalimantan Province has a total area of about 37 530.52 km^2 or 6.98% of the island of Borneo and 1.96% of the total area of Indonesia. Not only have optimal land but also have sub-optimal land such as wetlands and dry land. Potential dry land in South Kalimantan also large around 190 442 ha. (BPS South Kalimantan. 2013). While the dry land in Indonesia generally have the ability to store water is low. Shallot crop requires irrigation sufficient. In addition to water deficit, according to Sumarni *et al.* (2006) Another problem that is often found in arid lands is the erosion of top soil (soil erosion) and the leaching of nutrients due to the flow of water on the surface. Increased soil moisture to support plant growth, among others, can be done by using mulch. The use of mulch in crop cultivation can serve to inhibit run-off and erosion (Anwarudinsyah *et al.*, 1993). The use of plastic mulch in Indonesia has been applied to vegetable crops. Mulching can help prevent water loss in the dry season and

prevent the accumulation of water in the root zone at the time of the excess water or rainy season. The water that infiltrated into the soil the plants can be used to increase plant productivity. On dry land, mulch also prevent solar radiation reaching the ground so as to reduce the evaporation of soil.

Mulching is a substance or material that is used to cover the surface of the land or agricultural land with the specific purpose of the principle is to increase crop production. Shallot crop farmers generally try with a system of ditches or making system water channels and face many obstacles, especially bio-physical constraints of land, climate and socio-economic as well as appropriate technologies. The influence of solar radiation, water availability and weeds is one of the limiting factors in the growth of the shallot. Shallot as well as vegetable crops has higher economic value but require more cultivation techniques and highly susceptible to environmental conditions are unfavorable. Therefore, specific technologies for the management such as land arrangement, amelioration, selection of commodities and water quality improvement is needed.

The purpose of this study was to determine the effect of the interaction between the use of black silver plastic mulch and shallot varieties on the growth of shallot in dryland South Kalimantan.

2. Materials and Methods

The study was conducted on farmer's land in April - September 2015 in two districts, there are Tanah Laut District, subdistrict Bajuin, Kunyit village and municipality Banjarbaru, Landasan Ulin sub district, Karangrejo village, South Kalimantan Province. Using a spacing of 17 cm x 17 cm, while the fertilizer used is chicken manure = 10 tonnes ha^{-1} that has been fermented, NPK fertilizer = 600 kg ha^{-1}, SP-36 = 100 kg ha^{-1} and KCL = 300 kg ha^{-1}

Chicken manure and SP 36 are given after land preparation and after liming. A half of NPK fertilizer dosage and a half of KCL dosage are applied at 10 days after planting. Then, the other dosages of NPK fertilizer and KCL fertilizer are applied at 20 days after planting.

Location 1: Kunyit Village Bajuin subdistrict, Tanah Laut District

Research was arranged in Randomized Group Design with 2 factors : the first factor is shallot variety, there are 8 varieties namely Bima Brebes, Sri Kahyangan, Rubaru, Biru Lancor, Super Philip, Bauji, Crok Kuning, Keta monca. The second factor is the use of mulch that is without mulch and with mulch.

Assessment plots is 8 varieties x 2 treatments x 3 replications x 20 m^2 = 960 m^2.
The total area is (100/65) * 960 m^2 = 1476.9 ≈1.500 m^2.

Location 2: Karangrejo Village, Landasan Ulin Subdistrict, Banjarbaru Municipal

Research was arranged in Randomized Group Design with 2 factors : the first factor is shallot variety, there are 8 varieties namely Bima Brebes, Sri Kahyangan, Rubaru, Biru Lancor, Super Philip, Bauji, Crok Kuning, Keta monca. The second factor is the use of mulch that is without mulch and with mulch.

Assessment plots is 8 varieties x 2 treatments x 3 replications x 10 m^2 = 480 m^2.
The total area is (100/65) * 480 m^2 = 738.46 m^2.

Soil sampling for soil analysis was done twice, both before and after liming

3. Result and Discussion

Characteristics of soil

At both location Karangrejo Village and Kunyit village initial soil sampling conducted and analyzed in a laboratory to determine the soil conditions. Results of laboratory analysis is shown in Table 1. Soil conditions at the location indicated by the relatively acidic soil pH 5.75 (Village Karangrejo) and very acidic soil with a pH of 4.82 (Kunyit village). The low pH value of the soil at both locations requires the addition of lime to create a better physical condition. Very low organic C, N, P and K available provided very low to low. Based on the content of the sand-dust-clay from each location has the texture of sand.

Table 1. Results of laboratory analysis of soil at assessment locations while before liming

Chemical composition of soil materials	Kunyit	Remark	Karangrejo	Remark
pH H_2O	4.82	Very acidic	5.75	acidic
C –Organik (%)	1.91	Low	1.269	low
N_2 (cmol (+)/kg)	0.286	Medium	0.201	medium
P Bray 1 (ppm P)	116.487	Very high	54.340	high
Ca-dd (cmol(+)/kg)	9.004	Low	6.844	low
K dd (cmol(+)/kg)	0.767	Low	0.767	low
KTK (NH4OAc 1 M, pH 7) (cmol(+)/kg)	45.10	High	13.75	medium
Na dd (cmol(+)/kg)	0.143		0.233	
Mg dd (cmol(+)/kg)	2.079		0.970	
KA (%)	3.27		2.86	
Texture : Sand (%)	43.72	Sandy silty clay	42.49	Sandy silty clay
Silt (%)	32.70		31.48	
Clay (%)	23.59		26.03	

Source : Soil Laboratory of Indonesian Swampland Agricultural Research Institute

After liming, there was done soil analysis by pH meter tool. Result showed that soil pH is on medium scale

Planting conditions

Based on the analysis of variance (Table 2) noted that the data the percentage growth and harvesting time did not differ significantly between the varieties and treatment (with mulch and without mulch).

Table 2. Result of variance analysis for interaction of mulch and varieties on shallot growth and yield

Source of Variance		Observation Variable					
		growth	harvesting	Diameter bulb	Number bulb	Weight per clump	Productivity
Varieties (V)	Banjarbaru	ns	ns	**	**	**	**
	Tanah Laut	ns	ns	**	**	**	**
Mulch (M)	Banjarbaru	ns	ns	*	**	**	**
	Tanah Laut	ns	ns	**	ns	**	**
V x M	Banjarbaru	ns	ns	*	**	**	*
	Tanah Laut	ns	ns	**	**	**	*

Remark : ns = non significant; * = significant ; ** = very significant base on DMRT test

Average of shallot growth percentage between the varieties showed the numbers are not much different as shown in Table 3. As for all varieties also have a uniform harvest time is 7 weeks after planting.

Table 3. Percentage of shallot growth and harvesting for shallot that planted using mulch and without mulch in Banjarbaru Municipality and Tanah Laut District

Varieties	Treatment	Municipality Banjarbaru		District Tanah Laut	
		% growth	Harvesting	% growth	Harvesting
Bima Brebes	Mulch	97	7	97	7
	Without mulch	97	7	96	7
Super Philip	Mulch	98	7	96	7
	Without mulch	98	7	95	7
Biru Lancor	Mulch	97	7	98	7
	Without mulch	97	7	97	7
Bauji	Mulch	95	7	95	7
	Without mulch	97	7	97	7
Sri Kayangan	Mulch	96	7	97	7
	Without mulch	96	7	96	7
Keta Monca	Mulch	95	7	95	7
	Without mulch	96	7	96	7
Rubaru	Mulch	97	7	97	7
	Without mulch	97	7	97	7
Crok Kuning	Mulch	97	7	97	7
	Without mulch	96	7	96	7

Percentage range of shallot growth is around 96% - 98%. It indicate uniformity of seeds used, good uniformity of harvesting and seed tuber size. In this study, it is attempted to get homogene conditions to avoid data bias, that covered : uniform seedlings : size, age and origin. On the other hand, uniformity of seedling growth percentages also show that the eight varieties grown has the ability to grow uniform on dry land (Figure 1)

Figure 1. Super Philip and Rubaru performance with mulch and without mulch

Table 4 shown that there is a significant interaction between treatment mulch with shallot varieties. Planting shallot in Kunyit village, Tanah Laut by mulch have a bulb diameter larger than that planted without mulch except for varieties Sri Kayangan, Keta Monca and Crok Kuning.

Sri Kahyangan variety have no significant differences between planting with mulch and without mulch. Varieties that have larger bulbs are Bima Brebes, Sri Kahyangan and Biru Lancor.

By some advantages of mulching, shallot that planted with mulch have larger diameter than withouth mulch. Some of these bulbs diameter is also affected by the number of tubers produced. Shallot that produce fewer tubers have diameters larger bulbs and vice versa (Figure 2)

Table 4. Bulb diameter (mm) and bulb number of shallot that planted using mulch and without mulch in Banjarbaru Muncipality and Tanah Laut District

Varieties	Treatment	Municipality Banjarbaru		District Tanah Laut	
		Bulb Diameter (mm)	Bulb Number	Bulb Diameter (mm)	Bulb Number
Bima Brebes	Mulch	17.69 c	5.27 def	30.2 a	8.1 abc
	Without mulch	18.17 bc	6.28 cdef	16.6 efg	5.9 bcd
Super Philip	Mulch	21.28 ab	9.93 ab	24.8 bc	2.70 f
	Without mulch	18.01 c	3.31 f	20.0 def	4.9 def
Biru Lancor	Mulch	22.36 a	8.07 abcd	26.0 ab	8.0 abc
	Without mulch	23.16 a	7.28 bcde	15.0 g	6.6 bcd
Bauji	Mulch	20.9 ab	10.64 a	20.3 def	5.20 def
	Without mulch	23.17 a	8.92 abc	13.3 g	6.0 bcd
Sri Kayangan	Mulch	22.01 a	11.07 a	27.1 ab	9.7 a
	Without mulch	23.22 a	6.68 bcdef	27.1 ab	5.80 cde
Keta Monca	Mulch	17.51 c	6.13 cdef	20.9 cde	4.3 def
	Without mulch	18.83 bc	8.14 abcd	23.9 bcd	6.3 bcd
Rubaru	Mulch	20.70 ab	8.33 abcd	23.4 bcd	3.70 ef
	Without mulch	21.77 ab	4.63 ef	16.2 fg	6.0 bcd
Crok Kuning	Mulch	19.32 bc	8.06 abcd	25.2 bc	9.7 a
	Without mulch	23.25 a	4.37 ef	27.8 ab	8.2 ab

Remark : Number in the same column followed by same letter are not significantly different at 1% Duncan

Figure 2. Bulb weight of Super Philip with mulch (A), without mulch (B), Rubaru with mulch (C), and without mulch (D)

In Karangrejo village, Banjarbaru Mucipality, shallot that planted with mulch produce smaller bulb than planted without mulch because it produce more number of bulb than shallot planted without mulch. Number of bulb influenced weight per clump (Table 5).

Five of the eight shallot varieties, namely Bima Brebes, Super Philip, Biru Lancor, Bauji, and Rubaru that planted with mulch produce larger bulb diameter than planted without mulch so that the weight per clump also higher (Table 5).

From Table 5 it is known that grown shallot with mulch from both sites (Kunyit village and Karangrejo village) have higher productivity than planted without mulch. It is associated with some observations on the previous parameters that the shallot crop that has a high weight per clump it will generate higher productivity value of shallot that has a lower weight per clump. Interconnected between varieties, bulb diameter, weight per clump and productivity.

Mulch reduce water evaporation from the soil around the roots of shallot mainly to dry land. As is known from laboratory test results that the texture of the soil in the study site in the Kunyit village, Tanah Laut District is dominated by sand texture. According Sumarni *et al.* (2006) in addition to the other problems of water deficit that is often found on dry land, namely the erosion of top soil (soil erosion) and the leaching of nutrients due to run off. Sand has properties that are less able to store water. Therefore, the use of mulch, the evaporation can be reduced. It is profitable for crops that planted in dry sandy soil texture because shallot requires enough water for the tubers growth.

Solar radiation, water availability and weeds are limiting factors of shallot growth. Mulching is one way to reduce water evaporation and reduce weed growth. Mulching is the material used on the surface of the ground and serves to avoid water loss through evaporation and suppress the growth of weeds (Adisarwanto and Wudianto, 1999 in Mariano., 2003). Anwarudinsyah *et al.* 1993 said that in addition serves to reduce runoff, mulch also serves suppress the growth of weeds, improve soil structure, increase soil's capacity to retain water, pore aeration and infiltration, and maintain the content of organic material so that the productivity of the land is preserved (Kadarso 2008; Arsyad 2010). Mulch can help prevent the loss of water in the dry season and prevent the accumulation of water in the root zone when excess water or rain. The water that infiltrated into the soil can be used plants to increase plant productivity. Besides mulch may block solar radiation reaching the ground so as to reduce soil evaporation. Soil infiltration and evaporation is a process that determines the availability of soil water on dry land. According to Ghuman and Sur (2001) mulch can reduce soil bulk density on the surface of the ground while the organic material can be increased due to the decomposition of the mulch. While Mukherjee et al (2004) said that black and white plastic mulch to maintain soil moisture and increase soil temperature.

Table 5. Weight per bulb (gram) and weight per clump for shallot that planted using mulch and without mulch in Banjarbaru Muncipality and Tanah Laut District

Varieties	Treatment	Municipality Banjarbaru		District Tanah Laut	
		Productivity (t/h)	Weight/clump (g)	Productivity (t/h)	Weight/clump (g)
Bima Brebes	Mulch	5.00 c	32.17 de	10.65 d	96.97 a
	Without mulch	1.03 c	21.01 e	7.9 ef	16.53 e
Super Philip	Mulch	13.13 ab	76.8 a	22.47 ab	46.53 cd
	Without mulch	7.6 bc	9.81 f	8.82 def	19.37 e
Biru Lancor	Mulch	6.23 bc	57.77 b	17.57 bc	70.45 b
	Without mulch	3.3 c	39.75 cd	3.57 ef	15.92 e
Bauji	Mulch	11.9 ab	56.36 b	16.3 cd	63.73 bc
	Without mulch	6.1 bc	43.31 c	2.08 f	22.17 e
Sri Kayangan	Mulch	5.3 c	50.27 b	26.4 a	78.1 b
	Without mulch	5.4 c	39.68 cd	14.47 cd	18.4 e
Keta Monca	Mulch	15.4 a	19.13 ef	10.63 de	22.43 e
	Without mulch	1.9 c	31.61 de	8.30 def	11.17 e
Rubaru	Mulch	9.1 b	35.94 cde	9.33 def	62.57 bc
	Without mulch	1.57 c	24.4 e	2.70 f	21.26 e
Crok Kuning	Mulch	14.23 ab	29.06 de	14.27 cd	40.1 cd
	Without mulch	10.4 ab	28.77 de	7.5 ef	16.92 e

Remark : Number in the same column followed by same letter are not significantly different at 1% Duncan

Based on the Rachman research results *et al.* (2004), Puustinen *et al.* (2005), and Jordan *et al.* (2010), a layer of mulch will increase surface roughness so that slow the process may slow interception of rain and run-off so that the lower coefficient of run-off. Run off delays can improve rain water infiltration during rain events. According Arsyad (2010) mulch may inhibit run off thus reducing the speed of run-off and run-off transport capacity. Mulching planting aggregates can protect land from the destructive force of a grain of rain, increasing the absorption of water by the soil, keep the soil temperature, maintaining soil organic matter and

control the growth of weeds, so as to increase crop yields. Weeds can reduce crop yields because of the competition in making nutrients, water, sunlight, and living space in addition to the competition in making nutrients, weeds also issued alleopati compounds that can interfere with plant growth.

4. Conclusion

There was an interaction between the use of plastic mulch and shallot varieties for the weight per clump and productivity of shallot.In the assessment in the Kunyit village of Tanah Laut District , Super Philip varieties are grown using mulch has the highest productivity is 26.4 ton ha^{-1} while Rubaru planted without mulch has the lowest productivity is 2.7 ton ha^{-1}. In a study in Karangrejo Village, Banjarbaru Municipal Super Philip varieties are grown using mulch has the highest productivity is 15.4 ton ha^{-1}, and Bima Brebes varieties are grown without mulch has the lowest productivity is 1,03 ton ha^{-1}.

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Effect of *Trichoderma* and *Penicillium* Application (Isolated From Pine Rhizosphere) to The Shallot Growth

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Abstract

One of the problem shallots cultivation is an imbalance fertilization between chemical and biological fertilizers (manure), chemical fertilizers are usually applied in excess, causing less efficient absorption of nutrients. The large quantities of chemical fertilizer are deposited in soil, it has hazard effect to the human health and microbial soil population. Two of the beneficial fungus lived in the rhizosphere, like genus *Trichoderma* and *Penicillium*, has an important role and function in supporting the implementation of environmentally farming because *Trichoderma* and *Penicillium* are best known to stimulate the growth of plants. They are classified as plant growth promoting fungus. This study aims to determine the effect of *Trichoderma* and *Penicillium* applications on the growth of the Shallot. This research was conducted in Lembang, West Java, in June-August until 2016, using a completely randomized design with 8 treatments and repeated 10 times. The results showed that applications *Trichoderma* and *Penicillium* one week after planting with a concentration of 15 ml / plant have affected on plant height, whereas the number, weight and diameter of shallots bulbs have not shown any significant results.

Keyword: Shallot, Trichoderma, Penicillium

1. Introduction

Increasing demand of agricultural product cause excessive use of inorganic fertilizer worldwide. Therefore, use of inorganic and chemical fertilizer yields increase of crop product, but the large quantities of chemical fertilizer are deposited in soil, it has hazard effect to the human health and microbial soil population. Recently, trends in agriculture are environmentally /ecofriendly agriculture, application of plant growth promoting microbial could substitute the reducing of the use inorganic fertilizer as alternative to improve chemical and biological soil (Babu *et al*, 2015). Application of beneficial microbes in addition not only to supply the nutrient needs of plants, but also helped enhance crop growth by secreting IAA and siderophore production and other secondary metabolites.

Between different groups of microbes, fungi that inhabit in the rhizosphere, is an important part of a beneficial microbes. It has higher populatiuon than soil bacteria (Babu *et al*, 2015). Two genera fungus known exist on ubiquitous population are *Penicillim* and *Trichoderma*. *Penicillium*, filamentous fungi are widely used as producers of organic acid, these species generally has low concentration in rhizosfer and exist more at greater soil depth than other genus (Phuwawat *et al*, 2001). Some species of *Penicillium* are well known as a antibiotic producer but just a little is known of interaction between *Penicillium* with plant growth and even with other soil fungi. *Penicilium* posses abilitiy to solubilize rock phosphate by synthesize a number of phosphatases which are necessary to solubilize phosphates from medium containing bound phosphorus (El-azouni *et al*, 2008 and Yadav *et al*, 2011). They can substitute the plant requirement for phosphate partly or overall. Besides solubilizing of rock phosphate, some *Penicillium* species have proved to be able to leach iron from low grade ores,

an ability that may establish favorable micro nutritional condition for crop plant (Nicoletti *et al*, 2012).

As well as with *Penicillium*, the effect of Trichoderma application on plant growth and interaction with other soil fungi are still on investegeted. Contreras-Cornejo *et al* (2009) reported that some *Trichoderma* species could stimulate the growth of arabidopsis sprout because they have important role to produced auxin signal that stimulate plant growth. Aplication of *Trichoderma* to soil is an effective way to enhance the plant growth, because they have capability to secrete plant growth promoting substance including IAA (Radhakrishnan *et al*, 2012). Some *Trichoderma* strains that live in rhizosphere have been shown to have effects on plants directly, including increase their growth potential and nutrient uptake, fertilizer use efficiency, enhance rate of seed germination (Sharman *et al*, 2012). Studies have demonstrated that *Trichoderma* increases root development and crop yield. But no study has reported the effect on *Penicillium* and *Trichoderma* application on the growth of shallot. Based on that description, we are interested to conduct this research if *Penicillium* and *Trichoderma* have an influence to the shallot growth

2. Methods

This research was conducted in Lembang-Jawa Barat, on June – August 2016 by using Completed random design with 8 treatments repeated 10 times. Soil samples were collected from Pine rhizosphere at Lembang, *Penicillium* and *Trichoderma* were isolated using serial dilution. 10 grams of rhizospheric soil was transferred to a 250 ml Erlenmeyer containing 90 ml sterilized aquadest and shaken for 30 minutes 120 rpm. Serial dilution were made in 0.9% NaCl sterilized aquadest and 100µl aliquots were spread on PDA media (10^{-3} and 10^{-4}), subsequently incubated on room temperature for 3- 7 weeks, Penicillium and Trichoderma colonies that appearing on the medium were isolated and sub-cultured for application.

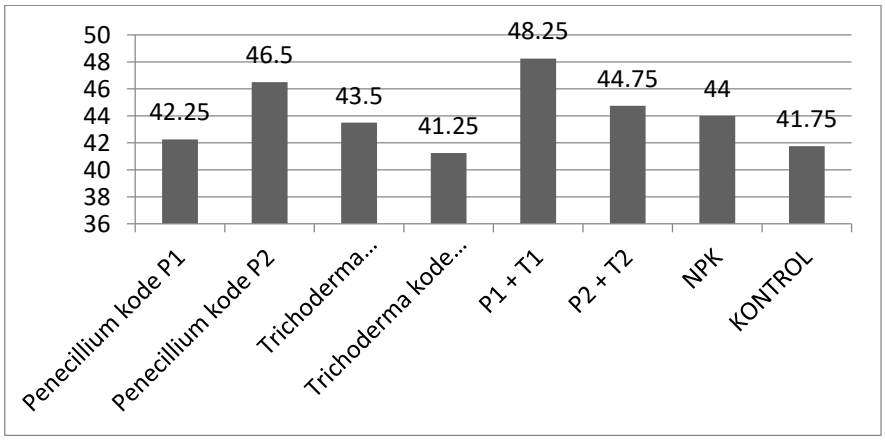
Treatments conducted in this research were *Penicillium* P1 (P1), *Penicillium* P2 (P2), *Trichoderma* P1 (P1), *Trichoderma* P2 (P2), P1 and T1 combination application, P2 and T2 combination application, NPK and control. Isolates to be tested have previously been purified on PDA agar media, subsequently cultured on sterilized corn media for 2 weeks.

Treatments was done by applying *Penicillium* and *Trichoderma* that were cultured on sterilized corn medium as much as about 250 gram and 2 weeks after inoculated, subsequently diluted as much as 5 liters water become suspension. The suspension was applied by watering right under shallot cultivation a week after planting as much as 15 ml per shallot plant.

3. Results and Discussion

The observed parameters in this study include: plant height, number and weight of saplings per clump, length and yields product. Parameter of plant height is measured at 6 weeks after planting (6 WAP) the results are shown in Table 1 below.

Chart 1. The average of plant height at 6 WAP



Based on the data in the above chart, almost all treatments give higher result compare to the control treatment. The treatment combination of P1 and T1 is able to increase plant height up to 15.56 percent. Single treatment of Penicillium is also able to increase the height of shallot even higher increase than the treatment of Trichoderma, it is hypothesized because penicillium applications can influence the growth of the shallot.

Treatment of P1 + T1 combination is the treatment with high result of the highest plant compared to all treatments with a result of 48.25 cm. In this treatment, there was occurring a mutually beneficial interaction between *Penicillium*, *Trichoderma* and the plants. *Penicillium* is assumed to have the ability of higher dissolving phosphate than Trichoderma, whereas Trichoderma produce higher IAA than Penicillium, so the application of both microbes is able to initiate cell lengthening of shallots and increase the plant height significantly. The other possible mechanism of growth promotion could be the ability of the fungus to colonize the roots and provide minerals to the plant on accessible form (Hossainet al, 2015)

The influence of treatment on the number, weight and diameter of shallot bulb is shown in Table 1. The results of this study shows that the number of clumps and weight of tuber on NPK treatment produce higher value than the other treatments, later followed by a single treatment of Penicillium P1 at second turn. Treatment of *Penicillium* P1 also shows the highest bulb diameter, while the combination treatment between *Trichoderma* and *Penicillium* treatments do not give influence to increasing of the number, weight and diameter of the bulbs produced.

Table 1. The influence of Penicillium and Trichoderma Treatments on the number, weight and diameter of the shallot bulbs

Treatments	Number of Bulb	Weight of Bulb	Diameter of Bulb
Penicillium code P1	10	36,25	2.40^{a}
Penicillium code P2	8,75	26,25	1.85^{ab}
Trichoderma code T1	7,75	16,25	1.80^{b}
Trichoderma code T2	7,5	18,75	1.80^{b}
P1 + T1	7,75	26,25	1.60^{b}
P2 + T2	8	30	1.90^{ab}
NPK	10,5	43,75	1.85^{ab}
CONTROL	10	27,5	1.90^{ab}

Penicillium treatment either single or in combination is estimated to influence the bulb weight that is generated, it is seen from the weights generated at the treatment is higher than the control treatment although it has not been able to compete with NPK treatment. *Penicillium* treatment also resulted in higher weight compared to the treatment of *Trichoderma.*

Applications of *Penicillium* and *Trichoderma* is expected could increase the available P in the soil consequently the P element that is needed by plants has been fulfilled, then the rate of photosynthesis that occurs in shallots can take place optimally, the result of photosynthesis is transferred to the formation of starch and protein in shallot bulbs, according to Rodrigues *et al*., (2003) content of shallot bulbs are carbohydrates, protein, fiber, minerals, sulfur and anthocyanins. Babu *et al* (2015) reported that inoculation with *P. menonorum* on cucumber plant significantly increased the starch and protein content on cucumber plant compared with control. Mechanism involve are increased of soluable P and enhancement of siderophore production that enhance photosynthesis and growth rate, which led higher protein and starch content in cucumber plant.

Penicillium application on shallot can enhance the growth through activity phosphate solubilizing and the secretion of IAA. Yadav *et al* (2011) reported that the ability of phosphate dissolving owned by *Penicillium* (301 ug mL-1) was significantly higher compared with *Trichoderma* (287 ug mL-1) measured after 6 days of incubation in media of pikovsaya broth, with the increase of mobilization of phosphate resources is insoluble and further enhance the uptake and siderophore production to increase the rate of photosynthesis and growth (Babu *et al*, 2015). Radhakrishnan *et al* (2012) reported that *Penicillium* PNF2 from peanuts rhizosphere significantly increase the height and weight of sesame seeds compared with controls, the filtrate culture analysis shows that PNF2 have the ability in the secretion of IAA with the highest concentration. Recent studies showed that discovery of the giberellins production by these fungi on large amount that explain the mechanism involved in growth-promoting effects on plant (Hossain *et al*, 2015). *Trichoderma* directly increases the P content in the soil, IAA and other plant growth regulator required by plant to support the growth on the vegetative and generative phases. Phosphate solubilizing fungus can meet partially or entirely requirements of phosphates in plants. Phosphate element is one of the macro elements that needed by plants to support growth in the vegetative phase, in order that when the P element is fulfilled then the weight of the plant will significantly increase (Fitriatin *et al*. 2009) and Goenadi *et al*. 2009).

The ability of each isolate also influenced the results of this research, it can be seen from the difference in the value of the test parameters on each single treatment. In addition to the ability of each fungal isolates with different test, the results of this research is also expectedly influenced by the concentration and frequency of application of the treatment, concentration which is not right and the number of applications that only once at the beginning of the planting has not been able to provide an increase in the quality and quantity of red shallots. It needs to do further testing on the concentration and frequency of application *Penicillium* and *Trichoderma* usage so as to optimize the production of red shallot.

By the time the research was conducted, it was scheduled to be observed on the intensity of plant phatogen, but the attack on the crop of shallots is not existed, either pests or phatogen, this may be triggered by environmental factors that are not supporting they to grow, so that the effect of using *Penicillium* and *Trichoderma* to the plant phatogen growth at the time of research was conducted cannot be reported.

4. Conclusion

Applications *Trichoderma* and *Penicillium* at one week after planting with a concentration of 15 ml / plant have affected on the plant height, whereas the number, weight and diameter of shallot bulbs have not shown any significant results.

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The Effects of Vernalization and Photoperiod on Flowering of Shallot (*Allium cepa* var. *ascalonicum* Baker) in Lowland Area

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Abstract

Flower development is a key element of seed production in plants. In Allium genera, this process strongly influenced by photoperiod and temperature. Photoperiod has been reported as important factor on shallot flowering in highland area, however the effect on lowland area has not been reported. This research was aimed to analyze the effects of vernalization and photoperiode on shallot flowering in lowland area. This experiment was carried out using local shallot varieties i.e: bulbs of Bima Brebes and Sumenep cultivars. The experiment was arranged in split plot design with 3 replication. The main plot was photoperiod with 3 treatments i.e: natural light (control), 2 hours night break, 4 hours extension using additional light while the subplot was vernalization of bulb seedlings with ambient (room temperature) and cold temperature (10°C) for one month. Vernalization increased the shallot flowering up to 11.68% in Bima Brebes cultivar and 2.85% in Sumenep cultivar. Photoperiod slightly decreased the percentage of flowering, but increased the bulbing ratio significantly. Both vernalization and photoperiod treatments decreased leaf biomass, leaf number and plant height. Vernalization effectively induced shallot flowering in lowland area, while photoperiod effectively induced shallot bulbs formation.

1. Introduction

Shallot (*Allium cepa* var. *ascalonicum* Baker) is a member of the genus Allium that closely related with onion (*Allium cepa* L.), which has a lot of bulbs in one cluster (ascalonicum) (Brewster, 1994). In Indonesia, shallot has been developed and cultivated by farmers, both in lowland and highland, because it is one of comodities that has high economic value as ingredient or food seasonings as well as for the food industry (Putrasameja dan Suwandi, 1996). However, this commodities price is often fluctuated in the national market because of unstability between supply and demand (Rachmat *et al*. 2012). This condition occured because the shallot production is only seasonal, which is generally planted in April or May, and July or August (Sumarni and Hidayat, 2005).

The attempt to increase shallot production has many constraints, especially due to seed shortage. Farmers usually still use bulbs as planting material considering the easier to use and has shorter harvest time than using true shallot seeds (TSS), though the allocation of production cost for supplying the bulbs seedlings was around 40% of total cost (Suherman dan Basuki, 1990). TSS is an alternative solution to solve this problem as it has several advantages, such as free of virus and seed borne diseases, not voluminous, reduce production cost of the planting material, healthier plants, and higher productivity than the bulb seedlings (Basuki, 2009).

TSS sometimes lacks the ability to flower naturally and producie seeds which results the average of flowering capability of shallot only 30% in highland area and even does not blooming in the lowland area (Putrasamedja and Permadi, 1994). True shallot seed production in Indonesia is still carried out in the highlands because it is an appropriate location to induce shallot flowering. The selection of planting sites will effect the productivity of seeds as the ambient temperature plays an important role in the success of shallot seeds production. In general, the highland is an optimum location to induce flowering

of shallot (Rosliani *et al*. 2013), while the lowlands are less suitable for initiation of shallot flowering (Putrasamedja and Suwandi, 1996). Vernalization treatment given to bulbs before planting may be the solution to increase the initiation of flowering in lowland area. The flowering percentage of shallots from Indonesia can be increased by storing bulb seedlings at temperature 4-10°C for 4 weeks (Rabinowitch and Currah, 2002). In onion, optimum temperature required to anable flowering is between 8-12◦C (Brewster, 2008).

Light is also one of important factor that affects flowering in the genus Allium. In general, shallot plants require photoperiod longer than 10 hours to bloom (Khokhar *et al.* 2007; Sopha et al. 2014). However, the research on the effect of photoperiod on shallot in Indonesia is still rare. This because Indonesia is located in equatorial region where the length of day and night is relatively similar all the year (Sutoyo, 2011). Sopha *et al.* (2014) reported that exposure over 10 hours on shallots on the highland area was able to increase flowering. However, there is no report about the effects of photoperiod on shallot flowering in lowland condition.

Photoperiod not only affects flowering in the genus Allium, but also affects the bulbs formation. Unfortunately, the study about development of shallot bulbs are still not much as in onion (Okubo *et al*. 1999). One of the environmental factors that affect the formation of bulbs was additional long day photoperiod (Rabinowitch and Currah, 2002). The ability of flowering and bulb formation of a plant is also influenced by genetic factors, so the physiological response of photoperiod and vernalization is different between varieties (Krontal *et al.* 2000). Therefore, it is necessary to test the influence of vernalization and photoperiod for inducing flower and formation of shallot bulbs in different varieties. This research was aimed to determine the effects of vernalization and photoperiod on shallot flowering in lowland area.

2. Material and Methods

The experiment was conducted at the Field Laboratory of Bogor Agricultural University in Ciomas, Bogor (06’ 36’’ 484°S, 106’ 47’’ 065°E) at altitude of 270 m a.s.l. from January to April 2016. Materials used in this study were bulb seedlings of shallot cultivars Bima Brebes and Sumenep with average of weight was around 5-7 g. Other materials were manure, NPK fertilizer (16-16-16) and dolomite lime. The equipments used were LED (Light-emitting diode) lamps 11 W (equivalent to a 100 W incandescent bulb), timers, cold storage, vernier caliper and thermometer.

The experiment was arranged in split plot design with 3 replications. The main plot was photoperiod with 3 treatments, i.e. natural light, 2 hours night break and 4 hours extension and the subplot was vernalization of bulb seedlings using ambient (room) temperature and cold temperature *(*10°C) for one month.

The natural photoperiod treatments was given by direct sunlight on the growing season as control. Four hours-extension treatment was applied similar as a natural treatment but given by addition of light exposure after natural light was gone (4 hours after sunset). Night break-treatment was given by additional light exposure at 23.00-01.00 (for 2 hours). Photoperiod treatment was given at the beginning of the vegetative phase, from 1 WAP (Week After Planting) untill harvest. Each unit consist of three pots size 25 cm with the volume of 4 kg soil, and each pot was planting three sets shallot seedlings.

The observed parameters were percentage of flowering, bulbing ratio, leaf biomass, plant height and leaf number. The data was analyzed using Analysis of Variance test (ANOVA) and followed by Least Significant Difference (LSD) at 95% significance level using STAR (Statistical Tools for Agricultural Research) software ver. 2.0.1.

3. Results and Discussion

Vernalization affected significantly on the percentage of flowering plants but not photoperiod treatment. In all photoperiod treatments only the plants that were treated by vernalization able to flower, while plants without vernalization were not able to produce flower, both on Bima Brebes and Sumenep cultivar (Figure 1).

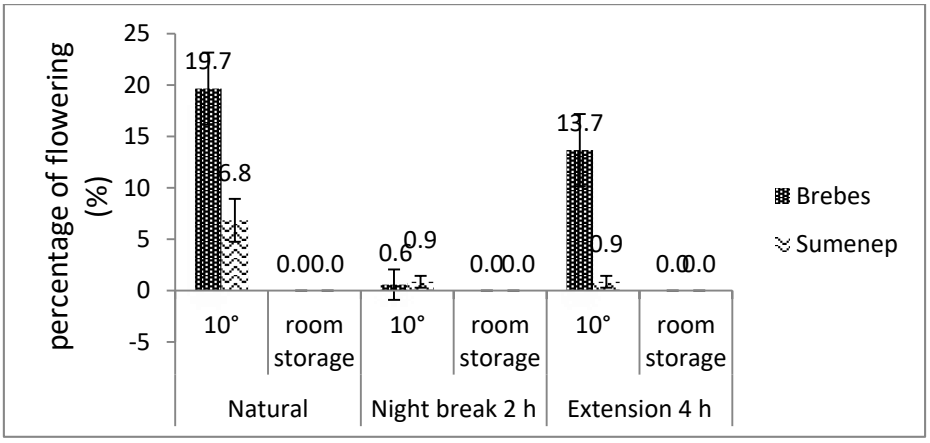

Figure 1. Effect of photoperiod and vernalization on percentage of shallot flowering

The data shows that the treatment using 4 hours extension and 2 hours night break had negative impact on flowering because those treatments even caused percentage of flowering lower than under natural conditions. In Bima Brebes cultivar, percentage of flowering in natural photoperiod was 19. 66%, whereas with 4 hours additional application it was 13. 68 % and in 2 hours night break it was only 1.71%. On the other hand, in Sumenep cultivar the percentage of flowering in natural photoperiod was 6.84%, whereas in extension it was 0.58% and night break it was 0.85%. The plants grown under natural or additional photoperiod without vernalization treatment were not able to flower (0%). The ambient temperature (without vernalization treatment) where the bulb seedlings was stored around 20-40°C has been known to be able to delay the inflorescence development (Krontal *et al.* 2000). This indicates that vernalization had stronger effect than photoperiod in inducing shallot flowering, and photoperiod did not able to replace the vernalization on shallot. In onion, vernalization was sufficient to induce flowering, while photoperiod and temperature advanced the inflorescence appearance, spathe opening and floret opening (Khokhar *et al.* 2007).

In this study, additional photoperiod significantly decreased the flowering ability. The reason behind this phenomen was not clear, since shallot is a long day plant (Sopha *et al*. 2014). The reason possibly related to the temperature of cultivation site. Thes cites of this research located in lowland area where the temperature average was around 25°C (Figure 2). This phenomenon had been reported via molecular study, that flowering was promoted by vernalization and correlates with upregulation of *AcFT2* gene, whereas bulb formation is promoted by *AcFT1* gene. Bulb formation is prevented by *AcFT4*. long-day photoperiods lead to the downregulation of AcFT4 and the upregulation of AcFT1, and this caused to promote bulbing.

Furthermore, the genetic factor also affected flowering ability. Flowering ability of Bima Brebes cultivar was significantly higher than Sumenep cultivar. Some reports suggested that Bima Brebes cultivar was one of shallots cultivar which was able to flower with low rate, while Sumenep cultivar was apparently very difficult to be flowering (Putrasameja and Suwandi, 1996).

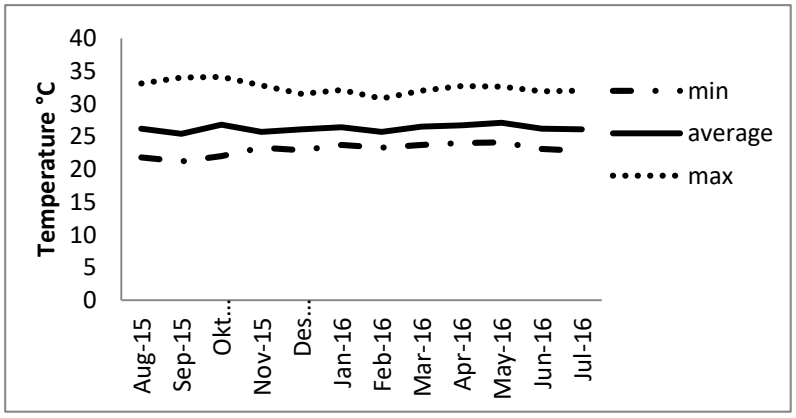

Figure 2. Environment temperature in 06' 36'' 484°S, 106' 47'' 065°E at altitude 270 m a.s.l.

Long photoperiod plus high temperature condition (25-30°C) caused degeneration of onion inflorescence within plant by competition of bulbing (Brewster, 2008). This fact was similar with the result of bulbing ratio in this experiment. The result showed that 2 hours night break increased bulbing ratio (Table 1). Bulbing ratio is ratio between maximum bulb diameter:minimum pseudostem. This ratio value could be used to observe the bulb formation with increasing in the ratio of bulbs (Okubo *et al.* 1999; Yamazaki *et al.* 2003; Brewster, 2008). Garner and Allard (1920), the first investigator, reported that long day photoperiods promoted bulbs development in onions. Further research showed that in long day condition, bulbing was faster in higher temperature (Brewster, 2008).

Table 1. The effect of photoperiod and vernalization on bulbing ratio

Treatments	6 WAP	7 WAP	8 WAP
Photoperiod:			
Natural	3.31 b	5.08 b	10.33 b
Night break 2 hours	4.37 a	7.84 a	13.46 a
Extension 4 hours	3.29 b	5.32 b	9.46 b
Vernalization:			
Without vernalization _Bima Brebes	3.26 b	5.39 b	10.54 a
Vernalization 10°C _Bima Brebes	3.97 a	7.00 a	12.05 a
Without vernalization _Sumenep	3.12 b	4.91 b	9.88 a
Vernalization 10°C _Sumenep	4.27 a	7.03 a	13.00 a

There is not interaction between main plot and subplot. Figures followed by the same letter in the same column are not significantly different at LSD with a level of 5%. (WAP: Week After Planting)

Photoperiod and vernalization also influenced plant height, leaf number per cluster and leaf dry mass significantly. In photoperiod treatment, 2 hours night break caused those parameters was lower than both natural photoperiod and 4 hours extension, while in vernalization treatment plant height and leaf number per cluster in ambient temperature (control) was higher than that of vernalization in 10°C, both in Bima Brebes and Sumenep cultivar (Table 2). In 2 hours night break bulb formation probably caused degeneration of vegetative growth. Lancaster (1996) reported that bulbing terminated leaf production in onion. During the juvenile phase onions could not be induced to flower or bulbing until reach a certain critical weight or leaf number (Brewster, 2008). In addition to photoperiod, vernalization also decrease those vegetative parameters. In garlic, vernalization at temperature 5-10°C was able to increase the percentage of flowering but inhibited the growth of vegetative, such as plant height, pseudostem diameter, number of leaves as well as to increase the production of peroxidase and superoxide dismutase (Wu *et al.* 2016)

Table 2. The effect of photoperiod and vernalization on vegetative traits

Treatments	Plant Height (cm)	Leaf/cluster	Leaf dry mass (g)
Photoperiod:			
Natural	45.80 a	26.48 a	1.74 a
Night break 2 hours	42.44 b	17.68 b	1.07 b
Extension 4 hours	44.34 ab	24.08 a	1.52 a
Vernalization:			
Without vernalization _Bima Brebes	48.24 a	26.17 a	1.91 a
Vernalization 10°C_Bima Brebes	46.49 b	18.79 b	1.54 b
Without vernalization _Sumenep	42.01 c	27.05 a	1.27 bc
Vernalization 10°C _Sumenep	40.03 d	18.98 b	1.05 c

There is not interaction between main plot and subplot. Figures followed by the same letter in the same column are not significantly different at LSD with a level of 5%.

4. Conclusion

The results suggest that cultivation of shallot in lowland area needs vernalization treatment to induce flowering. Photoperiod did not able to replace the vernalization on shallot flowering in lowland area. Additional photoperiod even thightly decreased the shallot flowering ability, but increased the bulb development. Flowering ability of Bima Brebes cultivar was higher than Sumenep cultivar.

Acknowledgements

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Metabolite Changes in Shallot (*Allium cepa* var *aggregatum*) during Vernalization

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Abstract

The flower induction in shallot can be initiated by providing a vernalization treatments. The vernalization might affect the composition of metabolome. The metabolomic composition determines the physiological processes and functions of the plants and parts. Metabolomes can spatially define the structure of tissues and organs including flower. The objective of this research is to determine metabolite composition in four different growth stadia of bulbs with different vernalization treatment. GC-MS analysis detected 88 compounds that were different at different growth stadia of bulbs. The results demonstrated that phytol is a major compound that suggested corresponding to metabolite changes during vernalization with regard to bulbs growth stadia. The correlation analysis confirm that the differences of metabolite profile might play a key role in flowering initiation in shallot.

Keywords : Shallot, vernalization, flower induction, metabolomic, GC-MS analysis

1. Introduction

Shallot (*Allium cepa* var. *aggregatum*) is vegetatively propagated using its bulbs which spreads of contaminations and deseases to the next generation. Propagation by generative part experienced problems because some genotypes unable to initiate the flowering. In onion plants, flowering can be induced by providing vernalization treatment at 4-5^{o}C (Ami *et al* 2013; Elsiddig 2015). Transition of vegetative phase into a reproductive phase involves a number of biological and biochemistry processes of plants (Halevy 1990; Bernier *et al*. 1993). The changes occur during metabolite process can be an important indicator to show the status of plant growth and development.

The last decade has seen an enormous trend towards plant extracts such as essential oils, volatiles, and other compound released by the secondary metabolism of plants. Secondary metabolites that are formed during the process secondary metabolism can be measured by GC-MS analysis. The analytical strategy (GC-MS) used to analyze the volatile compounds, and selected compounds were structurally analysed by mass spectrometry transposing the method to GC-MS (Lekshmi *et al*. 2014). Metabolomic technologies have recently revealed new insights in biological systems through metabolic dynamics (Iijima 2014). Investigation on the biological activities of *Allium* compounds, as well as other phyto-compounds, and their mechanisms of action is still a major challenge for biochemistry, microbiology and plant breeding program. This study aims to determine metabolite composition in four different growth stadia of bulbs with different vernalization treatment.

2. Materials and Methods

Plant materials

Shallot bulbs of variety Bima Brebes were used as planting materials. Bulbs weight at 5-7 g were selected and germinated to shoot growth (1-3 cm in lenght). The treatments consisted of vernalization treatments, and noticed as non-vernalized bulbs (S_0), and vernalized bulbs at 3 growth stadia (S_1 = 1 cm of shoot, S_2 =2 cm of shoot, S_3 = 3 cm of shoot). The vernalization treatment were placed in chamber at 8^{o}C for 5 weeks. The bulbs were then planted in 45 cm diameter polythene bags containing 8 kg of growing media (soil: manure: husk = 2: 1: 1). Each polybags planted three bulbs. Before planting shallot bulbs soaked in a fungicide solution with the active ingredient benomil 50% at a concentration of 2 g/L for 15 minutes. Furthermore tubers soaked back into solution PGPR (plant growth promoting root) with a concentration of 5 g/L for 15 minutes. NPK fertilizers is given with a ratio of 15:15:15, a dose of 600 kg/ha or 2.4 g/polybag. The fresh leaves at 4 weeks after planting were carried directly to GCMS investigation.

GC-MS analysis

The samples were the leaves of shallot plants at 4 weeks after planting. GC-MS pyrolisis unit was carried out on an GCMS-QP2010 system coupled to Mass Spectrometer Detector. The sample were inserted into the quartz chamber in the pyrolysis unit. Helium was used as a carrier gas in a constant flow mode at 0.85 ml/min. The pyrolisis chamber were heated in an oxygen-free environment at a temperature of 400^{o}C for 0.2 minutes. The reaction will produce heat-mediated cleavage of chemical bonds in the macromolecular structure and produce low molecular weight with a chemical composition that identify specific compound of metabolite. Compound mixtures were then passed trhough the column GC-MS analysis. The column is Rt x 5 MS, with length 60.0 m, thickness 0.25 µm, and diameter 0.25 mm.

The initial temperature of the column was 50°C, which was gradually increased by 10°C up to 280°C. At the end of this period, the column oven temperature was 50 ^{o}C raised up to 280°C. Injection port temperature was ensured as 280°C and helium flow rate as 0.85 ml/min. Mass spectrometer detector was employed to detect compounds when they were vented from the column. Temperature of the detector was 200°C. The mass spectra obtained through GC–MS were analysed by using data library such as WILEY7.LIB and the NIST webbook database. The volatile compounds of the plant samples were then identified for each treatments.

3. Results and Discussion

The edible Allium are characterized by their rich content of thiosulfinated and other organosulfur compound. Metabolite composition in the shallot leaves extracts were identified by GC-MS analysis. The 88 metabolites were identified in this research.

GC-MS analysis of the shallot leaves in this research showed that the different of vernalization treatment contains different metabolite composition. In non-vernalized bulbs has the highest content of formamide (CAS) methanamide (37.00%). While in vernalization bulbs (with 1 cm of shoot) contains methanamine, N-methyl (CAS) dimethylamine (41.45%), butane (5.98%), and phytol (6.81%). (Figure 1a). Formamide is a clear liquid which is miscible with water and has an ammonia-like odor. Lokke *et al*. (2012) reported that in onion bulbs had the highest concentrations propanethiol and had an odor activity value 20 times higher than dipropyl disulfide.

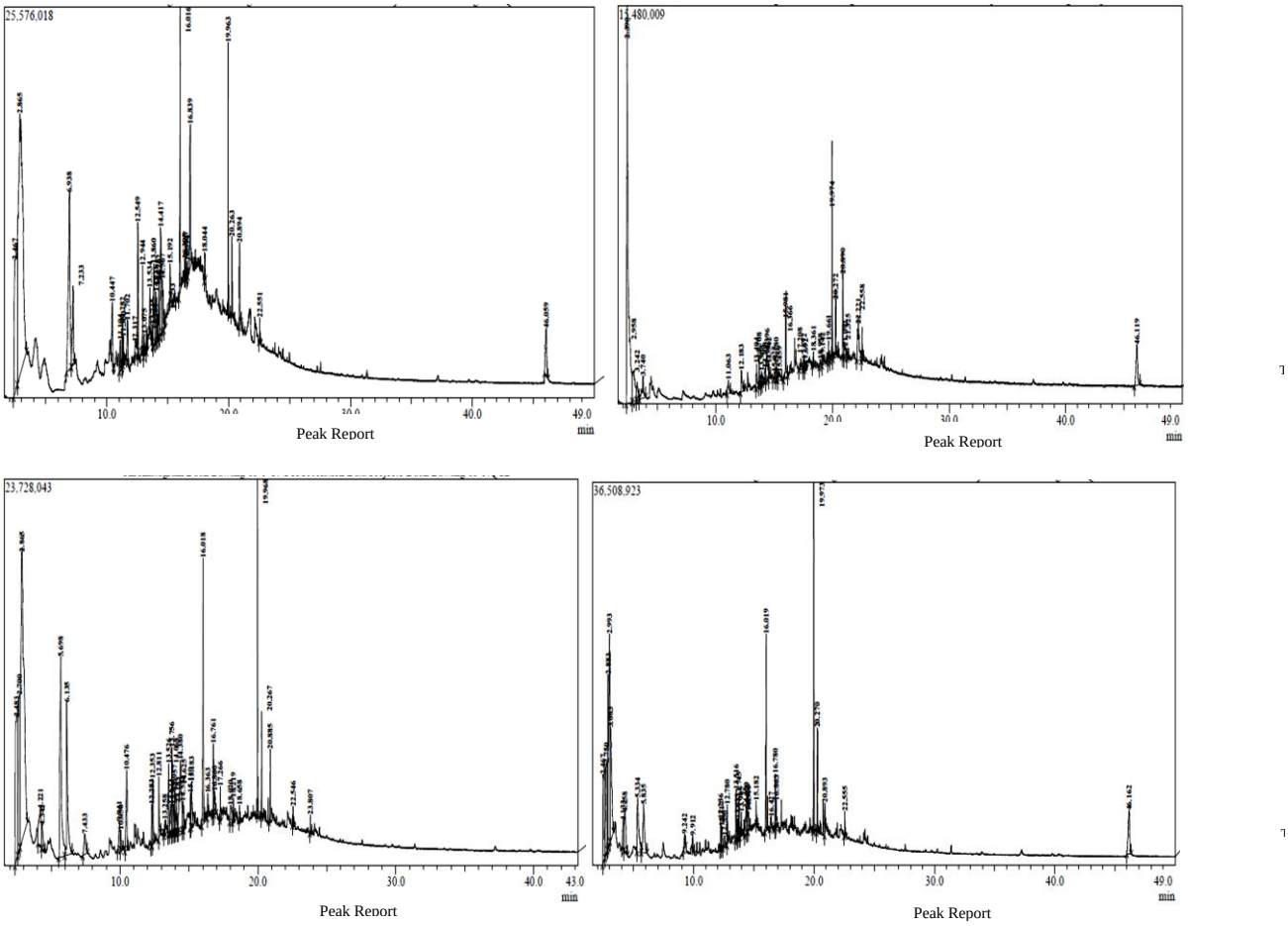
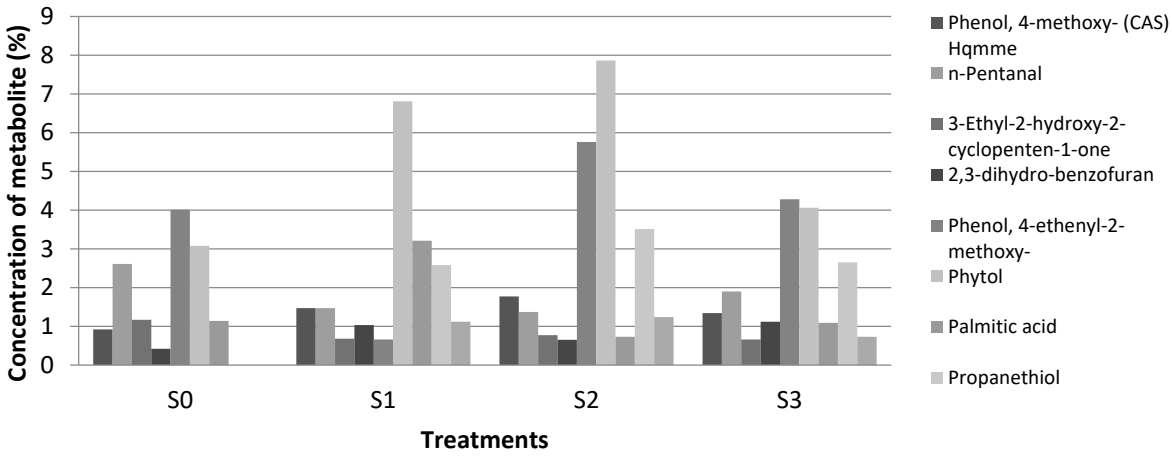


Figure 1. Metabolite changes in shallot. a) non-vernalized bulbs, b) vernalized bulb, bulbs stadia with 1 cm of shoot, c) vernalized bulbs, bulbs stadia with 2 cm of shoot, d) vernalized bulbs, bulbs stadia with 3 cm of shoot.



S_0 = non-vernalized bulbs, S_1 = vernalized bulbs, bulbs stadia with 1 cm of shoot, S_2 = vernalized bulbs, bulbs stadia with 2 cm of shoot, S_3 = vernalized bulbs, bulbs stadia with 3 cm of shoot

Figure 2. Metabolite composition in non-vernalized and vernalized bulbs of shallot (4 weeks after planting)

The results showed metabolite composition with different concentrations in all treatments. The content of phytol in vernalized bulbs were higher (4.28 to 7.86 %) compared with non-vernalized bulbs (4.01 %). Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. Percentage of flowering plants in non-vernalized bulbs was only 29.63% which is significantly different from vernalized bulbs (79.63%), *data is not shown*. Continued research in biochemical and physiological are needed to prove the role of phytol in flower initiation process of shallot.

The results was also revealed that metabolite composition in vernalized bulbs were higher than the non-vernalized bulbs. But, the content of n-pentanal and 3-ethyl-2-hydroxy-2-cyclopenten-1-one, in non-vernalized bulbs were higher than vernalized bulbs (Figure 2). Pentanal, also called pentanaldehyde or valeraldehyde, is an alkyl aldehyde, molecular formula $C_5H_{10}O$. It is used in flavorings, resin chemistry, and rubber accelerators (https://en.wikipedia.org/wiki/Pentanal). The results clearly showed that the odors and the flavours compound were identified higher in non-vernalized bulbs than vernalized bulbs of shallot. Most of the disulphides and thiol groups in onion were also determined by Lokke *et al*. (2012) and Lekshmi *et al*. (2014) determined the presence of ethanol, isoamyl acetate, isobutyl alcohol, propyl alcohol, palmitic acid, stearic acid, and lanosterol in the onion cultivars.

There are 7 metabolites composition were be found in each vernalization and bulbs growth stadia treatments with different level concentrations. The vernalized bulbs seemed had a higher concentration of metabolite compared to non-vernalized bulbs of shallot. There are also two compounds in vernalized bulbs but can not be found in non-vernalized bulb, namely 1-propanethiol (CAS) propanethiol and phenol, 4-ethyl- (CAS) p-ethylphenol. Propanethiol is an organic compound with the molecular formulas and structural formulas similar to alcohols, except that sulfur-containing sulfhydryl group (-SH) replaces the oxygen-containing hydroxyl group in the molecule. It is a colorless liquid with a strong, offensive odor (https://en.wikipedia.org/wiki/Propanethiol). Further study is recuired to prove a specific compound responsibility in flowering initiation in shallot. Mass spectrometry combined with a separation technique offers tremendous opportunities for analysis of complex biological samples because it enables the determination and identification of a large number of metabolites in a single analysis (Villas-Boas *et al.* 2005).

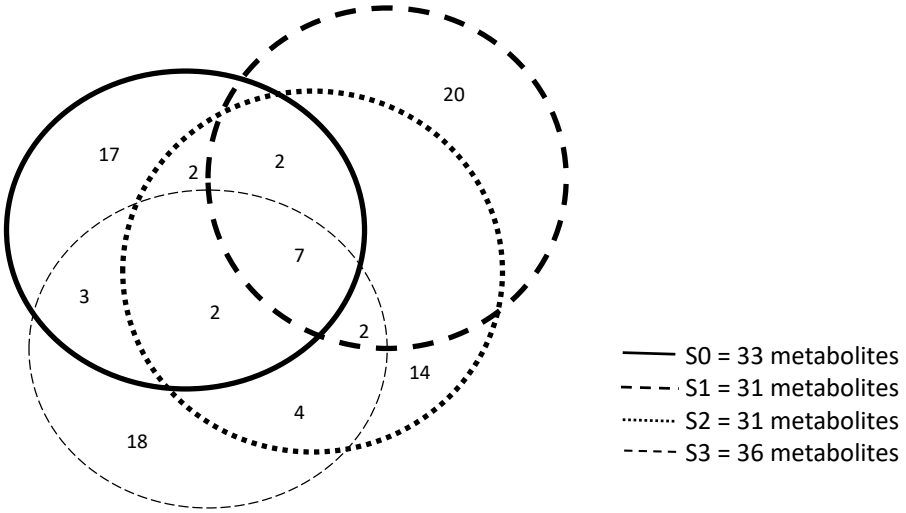


Figure 3. Metabolites distribution in non-vernalized and vernalized bulbs of shallot

4. Conclusions

The investigation metabolites compound in shallot by GC-MS revealed the dynamics of the emission of metabolites change during vernalization of germinated bulbs. By means of GC-MS, an estimate of the concentrations of different compounds in the freshly leaves of shallot was indentified. The results demonstrated that phytol is a major compound that suggested corresponding to metabolites changes during vernalization with regards to bulb germinated stage.

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Stakeholders Analysis in the Development of Seed Provision System Originating from True Seed of Shallot

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Abstract

True Seed of Shallot (TSS) is a new approach in the provision of shallot seeds. Through this technology some problems such as shallot seed borne diseases and transportation issues can be addressed. Previous studies suggest that the use of this technology can increase farmers income. However, contrary to the results, some of the farmers still consider that the use of the technology is difficult and takes more time compared with using a conventional seed. To develop this tehcnology, it is required the cooperation from several stakeholders that have interest in the provision of shallot seeds. This paper aims to analyze the stakeholders in the development of onion seed technology using TSS. Analysis of stakeholders will including Stakeholder Participation Matrix to identify the characteristics, interests, resources, problems, and attempt to do as well as using the Influence and Importance Matrix to determine the position of each stakeholders and efforts to improve their performance.

Keywords: True Seed of Shallot, stakeholders, Stakeholders Participation Matrix, Influence and Importance Matrix

1. Introduction

Shallot is one of the horticultural products that have important role in Indonesian economics. This commodity also known as a strategic commodity because it used for food seasoning and the fuction still can not be substituted by other spices commodities. related to the national economic condition, shallot well known as a contributor to inflation due to rising prices. In the raini season, the price will increase because at that time the land for shallot cultivation used to grow rice (ICHORD 2012). This problem will also affected to the shallot price that will follow the stock of the shallot in the market. This shallot price for consumption price also can give an effect to the price of shallot for seed. This case is very important because the main obstacle in increasing production and productivity of shallot one of them is because they still lack the availability of quality seeds, both in quantity and price. On the other hand, the seed is one of the largest cost component both in the farming of shallot (Nurasa and Darwis 2007).

Currently the shallot seeds used by farmers is generally an uncertified bulbs seed known as JABAL seed (Jaringan Benih Antar Lapang – Seed Networks Between the Farmfield in Bahasa Indonesia) derived from shallot for consumption or imported bulb seed. Farmers generally use the seeds derived from the harvested shallot for consumption from previous planting season or buying from other farmers (Basuki, 2010). But the problem is when the price of shallot for consumption is high, the farmers will also sell the shallot that prepared for seeds as shallot for consumption. If this happened there will be no seeds for upcoming planting season.

Imported seeds sometimes also imported shallot for consumption that used for seeds thus not qualified as well as certified seed. This things causes the seeds that used are the poor quality seeds that will causing low productivity of shallot. The use of shallot bulbs as a seed also has

several drawbacks including low shelf life, expensive transport costs, and often there is a seed-borne diseases.

The problems faced in the actual shallot seed provision system can be handled using the technology of shallot seed provison system throught the botanical seeds of shallot or well known as True Seed of Shallot (TSS). The use of TSS can resolve some of the shallot seed problems such as no additional time for seed dormancy, small volume that can simplify storage and transport, as well as free of the disease (Ridwan et al 1989). in the last few years Indonesian Agency for Agricultural Research and Development through the Indonesia Center for Horticultural Research and Development (ICHORD) and Indonesian Vegetables Research Institute (IVEGRI) has develop the technology of TSS for shallot seed provision.

Actually this technology is not a new technology because shallot can produce botanical seed naturally in certain condition. Better say that this technology are the new approaches in shallot seed provision system. The problem is the shallot farmers are not used to grow shallot from botanical seed as well as talked before that the farmers used to grow shallot from the bulb seeds. This problem is one of the weakness in development of TSS technology although the previous research stated that the use of TSS is more profitable than the use of bulb seed (Basuki 2009).

With all the advantages offered, TSS expected to answer all of the problems that exist in the shallot seed provision system to support the development of agriculture. The main problem of the TSS development are the mass production technique still not develop yet and also the technique for TSS utilization are still not sicialized to the farmers (Pangestuti and Sulistyaningsih 2011). In the development of the technology it is required the collaboratio between the stakeholders that involved. In an agribusiness system there are many stakeholders involved both directly involved in on-farm activities or in support activities. That many stakeholders will be associated with the activities of economical, cultural, and other social factors. This will lead to consequences that certainly will influence both in terms of cost, time, and the benefits to be obtained. It is required coordination among the stakeholders involved in order to achieve the expected goals which in this case is the development of technology TSS. The aim of this paper to analyze the stakholders role in developing the seed prvision system based on TSS technology.

2. Methodology

This paper is a part of the horticultural policy analysis in ICHORD at year 2016. Focuss of the problems in this paper is to analyze the role of each stakeholders identified in the development of seed provision technology based on TSS in order to obtain the formula to increase their role in seed provision system development based on TSS. A Focussed Group Discussion has been done to identify the stakeholders that will involved in the development of seed provision system based on TSS. Focussed Group Discussion will analyze the involvement of the stakeholders in the development of shallot seed provision system based on TSS.

The result from the discussion then transformed into the Stakeholders Participation Matrix to identify the role of each stakeholders, and also Importance And Influence Matrix to identify the position of the stakeholders. By identifying the position of stakeholders, we can take up a recommendation to determine which stakeholders that the role should be enhanced.

3. Result and Discussion

About True Seed of Shallot Technology in Indonesia

TSS technology is not really a new technology in the provision of shallot seeds. Previously this technology was introduced first time in 2009 by PT. East West Seed Indonesia, one of the seed company in Indonesia. PT. East West Seed with the brand "Panah Merah" introduce Tuk-Tuk shallot variety. But in the development this vareity not developed well due to its characteristics that less preferred by the farmers. In addition in the Tuk-Tuk sales are not introduced the gradually concept of seed provision. By direct seeding from the botanical seed to the shallot for consumption need more time if compared with producing shallot for consumption by bulbs. Direct seeding need about three months longer than shallot production with bulbs seed that only need two months.

Since 2012, IAARD through ICHORD and IVEGRI has develop this technology to solve the problems in common seed provision system. Many research has been conducted to develop this technology in past few years (Sopha *et al* 2014; Sopha and Basuki 2010). A seed provision system also has been introduced which is a gradually system that make the shallot farmers do not have to grow shallot from the botanical seeds, because the farmers are not used to cultivating shallot from botanical seed directly. In this system, the botanical seeds will be planted to produce mini bulbs. The farmers will use the mini bulbs as seed in their shallot production.

Stakeholders analysis in the development of seed provision system based on TSS

Multi stakeholders process is a collaboration between actors that related in one dialogue process and action to solving some problems. Characteristics in multi stakeholders approaches are (1) involving some actors that collaborate for one purpose, (2) focussed on the purpose for change, (3) integrated bottom-up and top-down strategy, (4) involving decision making through collective rule and preocess, (5) working cross-sectoral and business, (6) bound in the structural changes system, and (7) the actors involved in the learning process (Stel *et al.* 2012).

As a new point of view technology in seed provision system, TSS utulization still limitid in some breeders and farmers that have connection with the technology owner such as PT. East West Seed from the private technology provider and IAARD through the ICHORD and IVEGRI from the government technology provider. Sumarni and Rosliani (2002) said that there are some some system that can be used to create the good quality seed of shallot such as direct seeding, seedlings production, and mini bulbs production. But the use of shallot seed originated from TSS will increase planting time, because the farmers usually use the shallot seed in bulb forms (Darma *et al.* 2015; Sopha and Basuki 2010).

From the selection shallot bulbs for seed, we can grow them until producing flowers and true seeds that will called True Seed of Shallot (TSS). From the TSS there are three ways in producing bulbs shallot seed or shallot for consumption. As Sumarni and Rosliani (2002) has explain the first way is directly planting the TSS in the fields to produce shallot for consumption. The second way is by producing mini bulbs and then the mini bulbs replanting to produce common shallot bulb seed, then the bulb seed will be use for producing shallot for consumption. The third way is producing shallot seedling to produce mini bulbs, seed bulbs or even shallot for consumption.

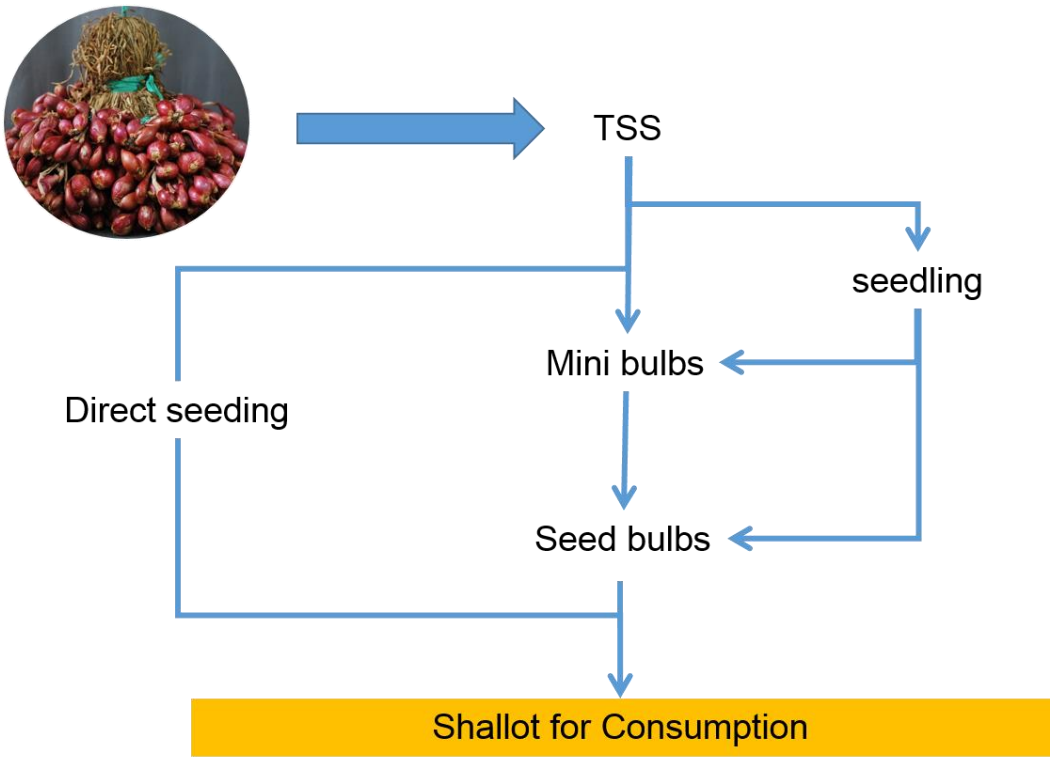

Figure 2. Shallot production system originated from TSS

From the identification there are some stakeholders that can involved in shallot seed provision based on TSS. There are 12 stakeholders from many institution with their own characteristics, importance, resources, problems, and also action needed to improve their role in shallot seed provision based on TSS.

Table 1. Stakeholders Participation Matrix in shallot seed provision system based on True Seed of Shallot

Actor	Characteristics	Importance	Resources	Problems	Action Needed
Directorate General of Horticulture	- National level policy makers - have a program and budget for development	Maintain stability of shallot supply and price	- Budget - Power	Shallot still seen as the secondary commodity	Utilize the TSS technology to develop shallot seed provision system
ICHORD/ IVEGRI	- Shallot seed technology provider - Owner of TSS technology	Disseminating seed provision technology	- Human Resources for mentoring - Budget allocated	Research fund still lack because of shallot still seen as the secondary commodity	Collaboration with private sector, association, and major shallot farmers
ICATAD/ AIAT	- Agricultural extention under the IAARD	Disseminating seed provision technology	- Human Resources for mentoring - Budget allocated	Research and dissemination fund still lack because of shallot still seen as the secondary commodity	Collaboration with local government, private sector, association, and major shallot farmers

Actor	Characteristics	Importance	Resources	Problems	Action Needed
Local Government	Local program to increasing shallot production	Run the local program	- Local budget - Extension services - Seed production facilities	Lack of competent extension services	Training of Trainers (TOT) for local extension workers
Breeder Group	- a group consisting of several breeder	Increasing group income through the provision of shallot seed	- land - capital - human resources	Lack of capital and competent human resources	- provide capital assistance - training for production
Major Breeder	More powerfull individual breeder	Increasing income through the provision of shallot seed	- land - capital	Lack of capital and competent human resources	- provide capital assistance - training for production
Seed Company	Provider of seed	Increasing income through the provision of shallot seed	- land - capital	Lack of competent human resources	- training for production
Shallot Farmers	User of shallot seed both botanical and bulb seed	To get the best quality seed	- land - capital	Lack of capital and competent human resources	- provide capital assistance - training for production
Shallot Association	Shallot agribusiness organization	- increase members income - get the best quality seed	- land - capital - human resources	Lack of competent human resources	- Training of Trainers (TOT) for shallot association
Seed certification agency	Institution under the provincial government that supervise the seed circulation	- To make sure the certified seed circulated are the good quality seed	- Seed inspectors	- The number of seed inspectors are still few - Seed inspectors still dont understand about the TSS technology	- Training for the seed inspectors
Extension agency	Institution under the local government that supervise the seed circulation	- Assisting farmers in agricultural development	- Human resources	Lack of competent human resources	- Training of Trainers (TOT) for organization extension
University	Have lot of resources in research and development	- To create, develop, and disseminate the technology	- Human resources, researcher, budget	Lack of information about the technology	- Collaboration with ICHORD and ICATAD to develop TSS technology

From the table above we can see that each stakeholders have their own characteristics, importance, resources, problems, and also action needed to improve their role in shallot seed provision based on TSS. Some of the stakeholders have same characteristics, importance, resources, problems. But the action needed are different depend on the caharacteristics of the stakeholders.

Directorate General of Horticulture, Indonesian Agency for Agricultural Research and Development (IAARD), and Indonesian Center for Agricultural Technology Assesment and Dissemination (ICATAD) are the institution under the Ministry of Agriculture coordination. Those three institution can together solve the problems in shallot seed provision system using the TSS technlology. But the problem is shallot still seen as the secondary commodity compared with the food crops commodity. Even though the shallot is one of the Ministry of Agriculture priority commodities, the fund that allocated for shallot development is still insufficient.

To identify the position of each stakeholder Influence and Importance Matrix are used. This matrix will place one stakeholder based on it's characteristics. Stakeholders will be divided into four quadrants in a matrix where the first quadrant is a place for stakeholders who have high importance but low influence, the second quadrant is a place for stakeholders who have high importance and high influence, the third quadrant is a place for stakeholders who have low importance and low influence, the fourth quadrant is a place for stakeholders who have low importance but high influence.

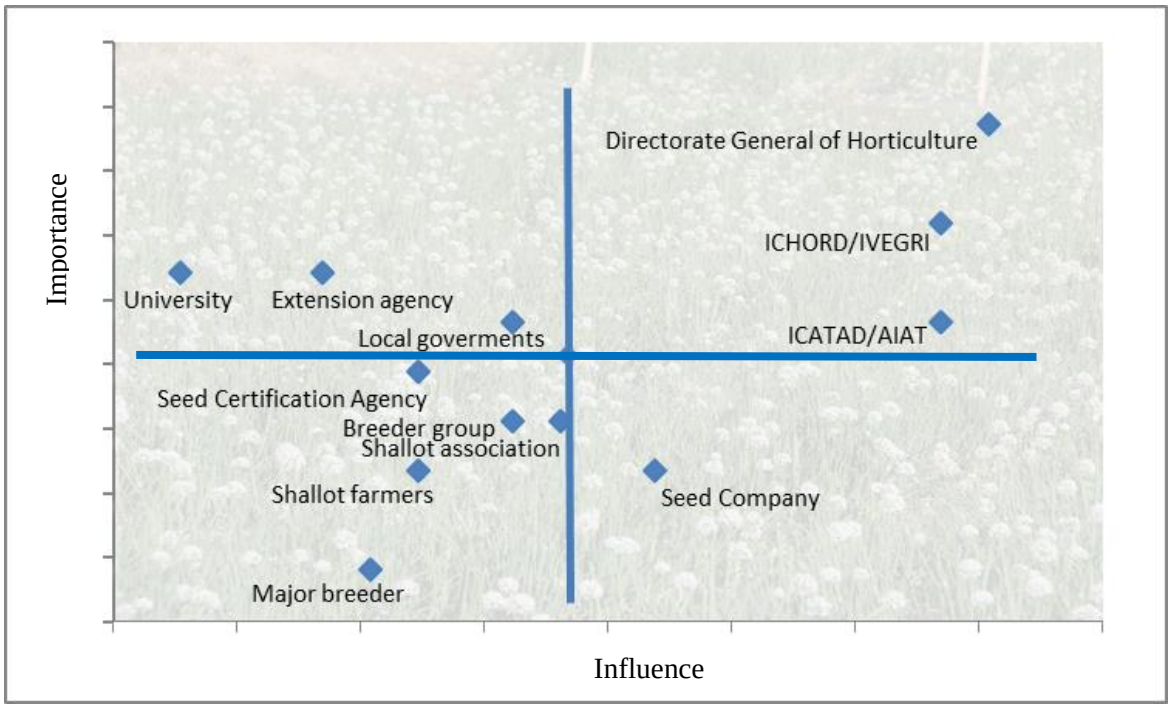

Figure 2. Influence and Importance Matrix in shallot seed provision system based on True Seed of Shallot

From the figure above we can see that the Directorate General of Horticulture, ICHORD/IVEGRI, and ICATAD/AIAT are the stakeholders with high importance and high influence. Those three stakeholders are the institution under the coordination of Ministry of Agriculture Republic of Indonesia. Directorate General of Horticulture is the institution that

have high power in horticultural policy including seed provision system. While ICHORD/IVEGRI and ICATAD/AIAT are the institution under the coordination of IAARD that have the duty as technology provider.

The stakeholders in first quadrant or have high importance but low influence are university, extension agency, and local governments. From this result we can find that those three stakeholders must be improved particularly the capacity and capability because those three stakeholders have important role in developing the shallot seed provision system based on TSS. University, extension agency, and local government are the stakeholders that connected directly to the technology users.

4. Conclusion

Based on the Influence and Importance Matrix in shallot seed provision system based on True Seed of Shallot we can see that university, extension agency, and also local government must improve the influence in developing shallot seed provision system based on TSS. Of course, it is also must have sufficient support from other stakholders especially the stakeholders with high influence and power such as the Ministry of Agriculture.

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Policy Analysis on Shallot Stock Seed Program through The Botanical Seed (True Shallot Seed/TSS)

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Abstract

The main problem of development of shallot (*Allium cepa L. Aggregatum*) farming system is the stock of quality seed, both in quantity and price. The use of bulb as seed have many disadvantages such as low productivity and decline, need a large quantities (1-1,5ton/ha), expensive, vulnerable to disease, difficult to storage and distribution. One alternative to stock the shallot seeds are using the seeds from botanical seeds / true shallot seed (TSS). The purpose of this study is to conduct policy analysis program through the provision of TSS. The results of the study obtained information that the policy for TSS development set out in the Ministry of Agriculture Regulation of number 131/2015 on Technical Guidelines of Shallot Seed Certification. TSS technology have decrease the seed needs about 5-7 kg/ha, seed cost is cheaper, easier storage and distribution, increased crop productivity and pest free. The use of shallot bulb seeds require as much as 1 to 1.2 tons per ha with price of Rp. 20,000 per kg. By using of TSS, farmers only need 5-7 kg of shallot seeds for one hectare with price Rp. 1.5 to 2.0 million per kilo. Farmers were able to save the cost of providing the seed around 66-85%. The use of TSS able to increase the income of farmers is about 60 -70 million per ha and reduce seed cost as much as 50% compared to the bulb seed. The main problem to develop of TSS is related to the techniques for the mass production and commercialization.

Keywords : Policy Analysis, Shallot Seed, True Shallot Seed.

1. Introduction

Shallots is a one off strategic agricultural commodities that became one of the government's target of self-sufficiency in addition to rice, corn, soybeans, sugar, beef and chili pepper. Productivity of shallot in 2014 reached an average of 10.2 tons/ha, as much as 1,233 tons of national production and harvested area reached 120.7 thousand ha (BPS, 2014). In the period 2010-2014, shallot production increased 5.74% per year as a result of an increase in harvested area of 3.70% per year and the increase in productivity by 2% per year (Pusdatin, 2015). The center of shallot production is in Central Java, East Java, West Java and West Nusa Tenggara. These four provinces have contributed 86.24% of total national production

The scarcity of shallot supply caused by the un equality of distribution of production, both in function of space and time. Scarcity of shallot usually occurs in February and April, as a result of the decrease in production as a result of the rainy season. Shallots price fluctuations occur due to biological factors that are easily damaged (perishable); and ecological factors in Indonesia is two, where the rainy season into production constraints vegetables including

shallot. Shallot production that is seasonally cause shallot needs beyond the harvest season can not be produce, so that the necessary actions imports.

The main obstacle of shallot production was there are no guarantee the availability of quality seed both the quantity and quality throughout the year. In general, farmers plant shallots using tubers as for consumption or seed production of import. This make a poor quality of seed, this condition results in low of production (Balitbangtan, 2016). From year to year the availability of shallot seed quality can not meet the needs of farmers nationwide. The average availability of shallot seeds reached 15-16% annually (Directorate General of Horticulture, 2010). Seeing this phenomenon it is necessary efforts to provision of quality seeds, healthy and high production with sufficient volume and are available every season so that farmers can plant.

One effort to solve the problems on availability of shallot seeds, IAARD make a seed technology and cultivation through True Shallot Seed (TSS) (Puslitbanghorti, 2015). TSS technology in the form of botanical seeds which functioned as a seed, so easily distributed. The use of TSS is expected to address the problem of seed shallot in Indonesia because it can meet the supply of shallot in quantity and quality, more efficient use of seeds, longer shelf life, handling in warehouses and transportation easier. (Basuki, 2009, Permadi and Putrasamedja, 1991). One of the technical approaches performed in the provision of disease-free seed varieties quickly and economically is through the breeding of botanical seeds (TSS).

2. Methodology

The purpose of the study is conduct policy analysis program through the provision of shallot seeds botanical (TSS). This study is a review study using secondary data derived from the results of previous studies, BPS, ICHORD, Pusdatin and the Directorate General of Horticulture. Analysis performed included: TSS program through the provision of seeds, technology advantages TSS, TSS production cost analysis and policy recommendations. The analysis was done descriptively using tables and directed at the problem of seed TSS.

3. Results and Discussion

Stock Seed Programmed on Shallot Through True Shallot Seed (TSS)

Currently, the government has established a policy to the needs of shallot through increased domestic production, so that each province shall grow shallot (DG Horti, 2015). One of the policies taken by the Ministry of Agriculture in the provision of quality seeds shallot support self-sufficiency is through the provision of seeds TSS. This policy was embodied in the Minister of Agriculture Rule No. 131/2015 on Technical Guidelines on Shallot Seed Certification. The purpose of this policy is that the production of shallots seeds can be carried out through the certification of seeds in order to obtain seeds of shallot seed quality and sustainability.

One effort to meet the needs of quality of shallot seed can be developed in TSS, other than in the form of seed tubers. Given the climatic conditions in Indonesia are diverse and shallot seeds bulbs can not be stored in a long time, it is necessary to develop the use of true shallot (TSS) which can be stored in large quantities and for longer and do not need a large place. On the other hand , TSS seed is to be healthier and easier to be distribution.

Advantages and Constraints from TSS Technology

According to the research Pangestuti and Endang (2011) the use of TSS has many advantages, including: 1) needs a little seed. The use of seeds as the seed for an area of only 3 - 7.5 kg per ha were if use bulbs require 1-1.5 tons/ha. 2). The cost for seed procurement is cheap. With TSS the seed needs only 3 - 7.5 kg, the cost of providing the seed becomes cheaper. If the average price per kg of TSS Rp. 1,000,000, the cost for seed only about Rp 3,000,000.00 - Rp 7,500,000 (can save the cost of seeds amounted to 62.5% - 80%. 3). Seed storage easier. Not necessary building / large space for storage of seed for seed size is much smaller than the bulb. 4). The long life of seed is so flexible, it can be planted when needed. 5). Easy and cheap to distribute. With good packaging, damage during the distribution process is relatively very small, and 6). Variations of seed quality is low so it can produce the shallot with high productivity.

The research results from Sumarni et.all (2010) states that the production of TSS in the home screen can not produce flower and TSS tend to crop damage due to disease. In general environmental conditions that determine the success of seed production TSS is 1). Climate, with temperatures 16-18^{o}C , not a lot of rain, low humidity and windy. 2). Soil with a pH of 6-6.8 for seed production and 3) Weeds or other crops that are insect pollinators alternative towing.

In the Minister of Agriculture Degree no : 131/2015 stated that the classification of shallot seeds derived from the seeds, then his seed remains the same class with the class of seed origin. But if the planted seeds of tubers, then his seed would drop his class one level. According Pinilih (2015) the seed breeding of TSS grown from tubers conducted on some varieties (Trisula, Bima and Pancasona) obtained a breeding population of uniform, so the shallot seeds if grown from seeds will produce a uniform population.

Suwandi (2015) says that TSS technology has the advantages of vertical planting conditions, productivity will increase, free from disease and virus, seed volume and lower manufacturing costs and does not require any special storage facilities and transportation. While TSS production constraints include low flowering due to unfavorable environmental conditions and seed formation is lower as a result of the low pollen viability.

Cost Analysis of TSS Production

The stock of shallot seeds through methods TSS provides economic benefits higher. Basuki (2009) states that the use of seed varieties TSS Tuk Tuk increase crop productivity, impacting the increase in farmers' net income between 60-70 million rupiah per hectare compared with the Bima Curut variety. In addition the use of varieties TSS will impact on farmers' income increase by 47-57 million per ha compared to imported varieties of seed tubers Tanduyung, and an increase in revenues of 22-32 million rupiah per hectare than local varieties of seed tubers Bima Curut derived from their own storage

The main priority of production cost savings can be made through technological innovation seed known as True Shallot Seed/TSS. The use of TSS in addition to saving the cost of production also has the potential to higher than seedlings derived from tubers. Basuki (2009) says about the technical and economic feasibility of shallot cultivation technology with seeds shows that the use of TSS is technically feasible because it can increase the yield up to 2 compared to the traditional use of seed tubers. In addition TSS technology is also economically feasible because it can increase farmers' net income between 22-70 million seeds per ha compared to traditional bulbs. The use of TSS Tuk Tuk varieties are on the level of results and the highest net income increase is planting a single seed with a density of 150 plants/m2.

4. Conclusions and Policy Recommendation

Technology of TSS is one of the alternative stock of shallot seeds are healthy and quality has the potential to overcome the scarcity of shallot seeds. This technology will be able to save seed requirements, increase productivity, easy storage and transport. TSS seed production system is a breakthrough technology to improve the competitiveness of commodities and farmers' income. The development strategy can be site-specific and off-season.

The technology innovation of TSS is a breakthrough technology that has the potential outcome even considerable impact. Stock of shallot seeds through TSS is easily, bulk, and sustainable can solve the problem of scarcity of seeds after the off-season in February/March to April/May. The success of seed systems of TSS in the field is strongly influenced by the variety, tuber physiology, precision farming and the local climate.

The obstacle factors of development of this TSS technology is the need for specific environments (high area) for the development of TSS. TSS only suitable to be developed on a high area, so can produce the seeds. In addition TSS technology currently requires socialization because farmers are still accustomed to using the original bulb shallot seeds that are faster and more practice to be planted. Need readiness of human resources in terms of cultivation and processing of botanical seeds into a mini bulbs, as well as the institutional preparedness seed so that the seed production of TSS can be disseminated.

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The Dynamic of Shallot Production, Supply and Price after the Implementation of Horticulture Import Regulations

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Abstract

Shallot in Indonesia is one of important and strategic vegetable that is classified into main foods other than rice. Shallot influenced on Indonesia macroeconomic condition, which the increased of its price can increased inflation rate, therefore shallot trade get serious concern from the Indonesian government. The production of shallot is fluctuate so that affect to the fluctuation of its price, which affect negatively to consumers price. Import of shallot is the solution to stabilize its price, but sometimes the importation was not in proper time and quantity, then affect negatively to price on farmers gate. At the beginning, the purpose of import regulations are to arrange supply to the market in order to stabilized consumer price, and it was a technical barrier to protect the farmers especially to arrange time of import when the production of domestic shallot are high. In this paper we analyzed the affect of the implementation of horticulture import regulations to the dynamic of shallot production and price. We show that the regulation which was start implemented on early 2013 is effective enough to push the domestic production of shallot got higher, so that can release the import dependence and stabilize supply to the central market (Pasar Induk Kramat Jati), but have not been able to stabilized price on farmers and consumers gate, whereas the shallot price got higher after the implementation of horticulture import regulations

Keywords : shallot, import, production, price

1. Introduction

Shallots is a strategic commodity that plays a role in causing inflation, in 2013 a significant contribution to inflation is equal to 0.44% where it has become the highest among the other foodstuff (BPS, 2013). Inflation is due to the high increase in prices caused shallot production and fluctuating supply, thus the balance of supply is important. Although the production of shallot every year always had a surplus, but the condition is not a monthly surplus, where there is a shortage of supply in certain months, especially during the off season.

In the biggest shallot production areas such as in Central Java, the peak growing season is expected to occur in April through September, so that the peak of the harvest occurred nearly every year for 6-7 months, and concentrated between June and December to January. While the lessharvest occurs from February to May and November. The reason for many of the major production centers areas at certain times to plant other commodities besides shallots, such as rice, corn, chilli and sugar cane. Shallots requires quite a lot of land irrigation, so it is generally cultivated in irrigated paddy fields or rainfed areas. Former wetland rice and sugarcane crops in lowland farmers generally be the first choice for shallot farming in several production centers, such as in Central Java and East Java (Suwandi, 2014). That's why shallot production usually decrease when the field are planted by rice in the rainy season (Rajiman 2010)

In order to meet the needs of shallot during the off season, the Indonesian government has importedshallot since 1983. It was originally done to keep the stability of shallots supply and price, but it is feared that in the long-term theincreasedofshallot importation will affect the domestic price level (Ariningsih, et .al, 2004) and will lead Indonesia's dependence on imported shallots. Increased imports of shallots due to the consumption of shallots has increased along with population growth (household consumption), the food industry, catering, tourism, and others are made from raw shallot. But often these import policies do not at the right time. If the import is done at the moment in the shortage market supply, it will certainly help to stabilize the price that is not too spike. However, if the import is done when the shallot is being harvest it will be very harmful for shallotgrowers, becauseshallot imports are usually cheaper than the local shallots, which will cause prices of shallotat the farmers gate fallen, and sometimes got lower than the cost of production.

In order to achieve food self-sufficiency and protect farmers from price fluctuations, since the middle of 2012 to 2013 Indonesian government began to implement policies of imports of horticultural products. This policy aims to protect domestic farmers by considering the harvest schedule as well domestic production capabilities beforeimport. Shallot import restriction policy set out in The Regulation of the Minister of Agriculture No. 60/PERMENTAN/OT.140/9/2012 and Ministryof Trade Regulation No. 30/M-DAG/PER/5/2012concerning Import Recommendation of Horticultural Products or usually called RIPH (Rekomendasi Impor Produk Hortikultura). RIPH form of recommendation of amount and allocation of import quotas to registered importers, horticultural products which may be imported, time of import and the amount of import quotas (Erwidodo and Sayaka, 2015). With these policies, shallot importation can only be done if the importer had obtained import recommendation from the Ministry of Agriculture and Import Approval Letter from the Ministry of Trade. Government revised Permentan No. 60/ PERMENTAN/OT.140/9/2012 by issuing Permentan No.86/PERMENTAN/OT.140/8/2013, where the provision RIPH fresh horticultural products for consumption shallots are based on the reference price provisions. If domestic shallot prices below the reference price, the import will be stopped and if the domestic shallot prices above the reference price will be expensive to import. The rate of the reference price set by the Ministry of Trade, based on Ministry of Trade regulation No. 47/M-DAG/KEP/8/2013, shallot reference price is Rp 25,700/kg. In addition to regulation governing on imports of horticultural products, government issued Regulation No 43/2012 concerning quarantine measures and restrictions port of entry for regulating the reduction of the entrance of horticultural products from the outside into the country, from eight to just four ports entrance only (Erwidodo and Sayaka, 2013). The fourth entrance is the Port of Belawan (Medan), Port of Tanjung Perak (Surabaya), Port of Soekarno Hatta (Makassar), and Soekarno Hatta (Jakarta). While the port of Tanjung Priok (Jakarta) which was the greatest entrance was closed for imports of horticultural products.

These regulations to control the import of horticultural products including shallots, especially in order to be controled at harvest time. Hopefully, by the rules may empower shallot farming, where the shallot farmers receive incentives from the prices established thereby stimulating the growth of shallot farming, which in turn can create food self-sufficiency. The purpose of this paper is to analyze the dynamics of the import, production, supply, and shallot prices after the implementation of horticulture imports regulations.

2. Research Methods

The method used in this research is using secondary data available. Data of shallot production, and data of shallot import obtained from the Directorate General of Horticulture and BPS. Data import is used to import data shallots HS code 0703102900, to import data in

2011 and earlier and HS codes 0703102100 and 0703102900 to import data from 2010 to 2015 year. Data price national average in 2010-2015 was obtained from the Directorate of Basic Foodstuffs and Essential Goods, Directorate General of Domestic Trade, Ministry of Trade. While the monthly supply data shallot years 2010-2015 obtained from Pasar Induk Kramat Jati (PKIJ). The data analyzed descriptively using tables and graphs to show changes in production and prices after the implementation of horticulture import regulations. T-test is done to see if there was a difference conditions before and after the implementation of horticulture import regulations. Data prior to implementation the import regulationsis in the period of 2010-2013, while imports after the implementation the import regulations is data in period of 2014-2015. The timing on the basis of the assumption that the application of import rules effective at the end of 2013.

3. Result and Discussion

Dynamics of Shallot Import in 2010-2015

The volume of shallot imported occur every year between February and May, with the peak in February and March. At that time indeed shallot production declined due in several production centers have to plant other crops. Shallot are usually imported from India, Thailand, Vietnam, Malaysia, and the Philippines.

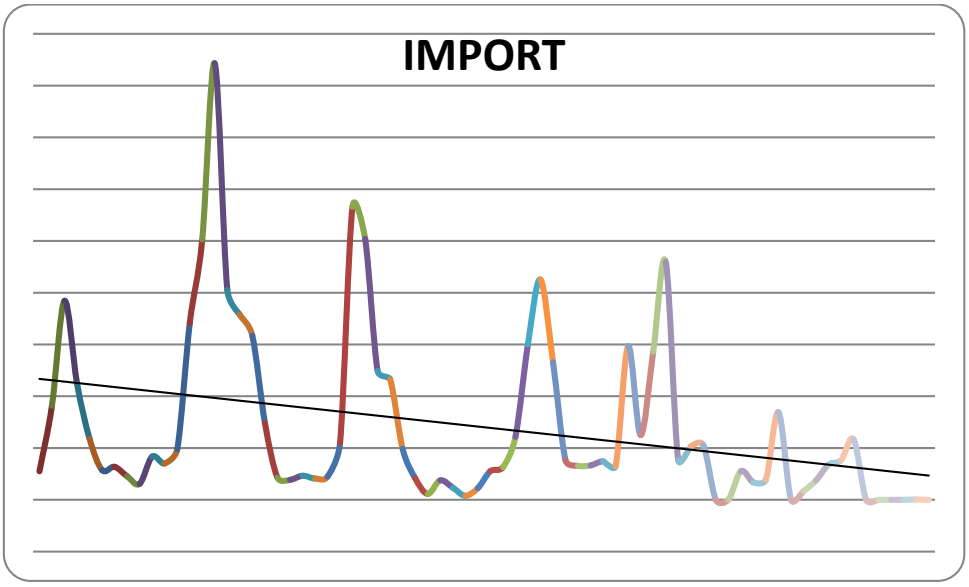

Figure 1. Dynamics of Shallot import in 2010-2015

In the period of 2010-2015 Indonesia still had a trade deficit of shallot or became net importer. In 2010 the volume of imports of 70.573 tons of shallot and then in 2011 jumped to 156.381 tons, an increase of 121%, which is the highest volume of imports. With the issuance of the Minister of Agriculture (Permentan) No.60/Permentan/OT.140/9/2012 on shallot import restriction policy, in 2013 the import of shallot is getting decreased, until in 2015 the volume of imports of shallots into 15.796 tonnes, or down 78.26% compared to 2014 (Figure 1).

Based on t-test results obtained average value of imports before the imposition of the import rule is 8.663 tons, and after the implementation of import rules into 3.685 tons, with a probability of 0.0011 (sig <0.05), thus the volume of imports before and after the implementation of horticulture regulations is significantly different or decreased significantly.

Dynamics of Shallot Production in 2010-2015

In the period of 2010-2015 period shallot harvest occurs around January, April to September, with the peak of the harvest in August. Conversely February, March, November and December are the months with the shallot production is low (Figure 2). This is because in several production centers such as Brebes, Cirebon, and Nganjuk which the land to grow shallots is a land similar to that used to grow rice, so that during the rainy season which usually occurs at the end to the beginning of the year, these lands are used for planting rice.

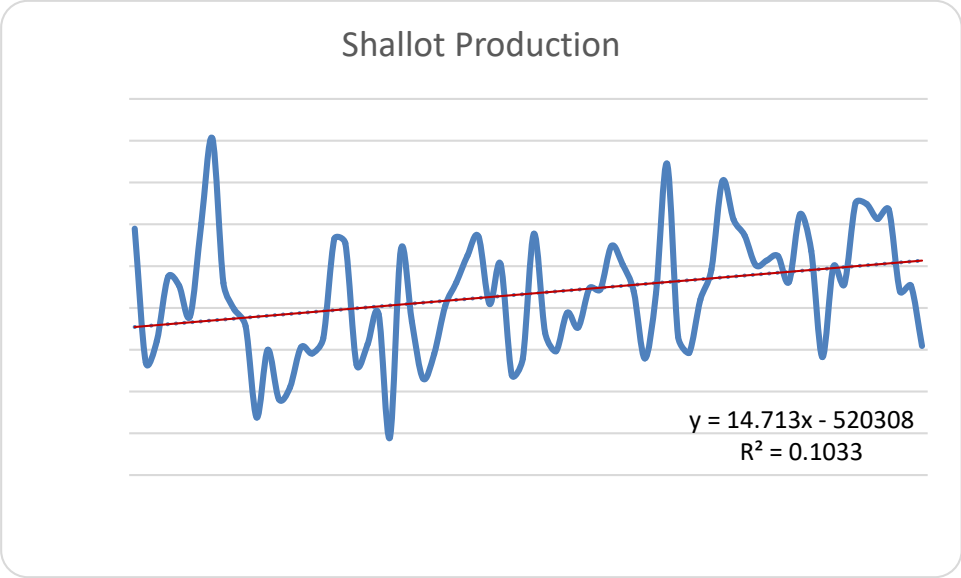

Figure 2. Dynamics of Shallot Production in 2010-2015

Shallots production 2010-2015 (last five years) generally increased, with an average growth of 3,93% per year. In 2010 shallot production in Indonesia from 1.048.934 tonnes to 1.233.984 tons in 2014 and represents the highest production during the period of the year.

From the t-test results showed the average value of production prior to the application of the rules of imports of horticultural products is 78.775 tons, and the average production after is 102.647, with a probability value 0.00029 (sig <0.05). Thus occurred the significant difference between the national shallot production before and after the implementation of horticulture importregulations. Therefore the import regulations has been able to push the production of shallot got higer.

The Dynamics of Shallots Supply at the Pasar Induk Kramat Jati (PKIJ)

Based on the graph in Figure 3 can be seen that the supply of shallot in Kramat Jati Central Marketor PIKJ always fluctuated throughout the year, this is because production is too volatile.

One effort to avoid fluctuations in the production of shallot is by setting appropriate cropping patterns and growingshallot planting areas (extension) to fill the supply shortage especially when the off season.

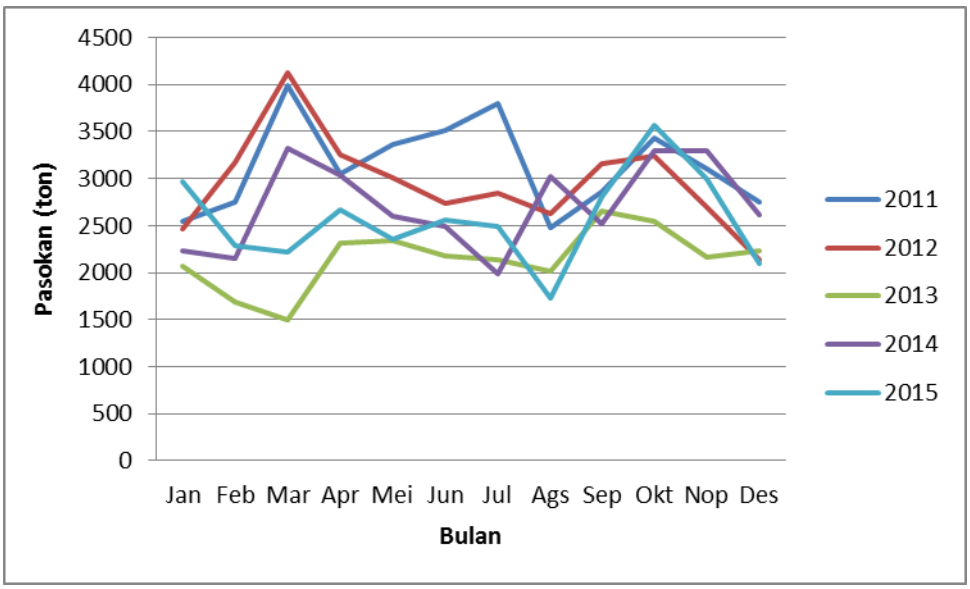

Figure 3. The Dynamics of Shallots Supply 2011-2015 in PIKJ

Based on t-test results before and after the implementation of horticulture import regulations showed the average supply before the implementation of the import regulations is 2747,72 and after the implementation of import regulations is 2638,33, with a probability value of 0.30 (sig <0.05), which means that the quantity of supply before and after the implementation of import regulations do not differ significantly. It can be concluded that efforts to stabilize supply through increased shallot production in the country has been successful.

The Dynamics of Shallots Price in 2010-2015

Based on the graph in Figure 4 can be seen that the trend of price movement 2010-2015 trend shows that almost equal, except in 2013 where there was significantly price increased in April and August. The increased of shallot prices was due to the declining production that affected the shortage in supply, coupled with the implementation of import regulations horticulture that has not run smoothly.

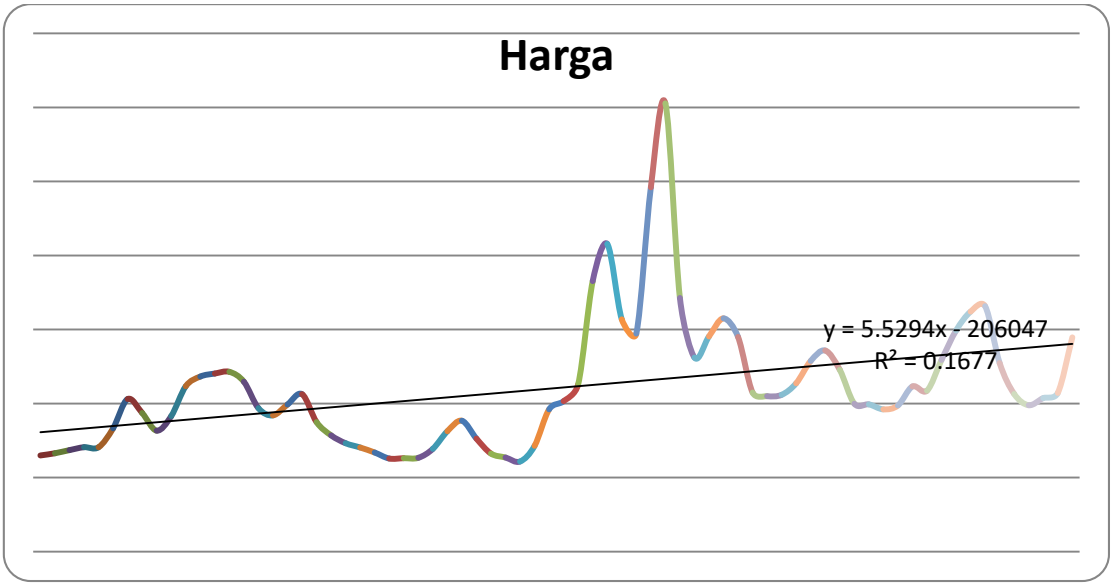

Figure 4. The Dynamic of Shallots Price 2010-2015

If the normal supply of shallot to PIKJ could reach 120 thousand tons per week, but in March-April 2013 supplies continue to fall up to 11 thousand tons per week. Every year in February and March, the supply of shallots from regions that are indeed declining. Therefore, the high rainfall makes shallot farmers switched to plant rice. In contrast to the previous years, the shift cropping patterns does not lead to price volatility due to a shortage of supply was immediately covered by imports, but after the import restrictions which did not run well lead to supply hampered. Economic theory suggests that price spikes occur when there is a shortage of supply were not able to compensate for the increase in consumer demand. The jump in prices will occur when there is a decrease in supply due to crop failure and / or policy failure (Erwidodo and Sayaka, 2014). Regulation of import restrictions were initiated in mid-2013 remains a bottleneck of discrepancies in the field, where the recommendations submitted at minimal production, but imports of shallots only realized at harvest time. Price fluctuations will occur and can't be avoided when the increase in consumption occurred outside the harvest season. The situation becomes more severe and more uncontrolled price spike when the government wants to achieve independence to tighten the import. Estimated production was overestimate coupled with estimated needs (consumption) which was less accurate and tend to underestimate resulted in the decision import quotas which also underestimate. This situation resulted in supply shortages and soaring prices of shallot (Erwidodo and Sayaka, 2014).

Based on t-test results obtained an average price prior to the implementation of import regulations is Rp 21.257,85, whereas after the implementation of import regulations Rp 23.948,68, with probability value 0,029 (sig <0.05). This showed that after the implementation of import regulations, shallot prices actually increased significantly. The increase in shallot prices is due to inflation that occurred in 2013 that caused the prices of production inputs got higher, especially on the components of seeds, fertilizers and pesticides. Seeds is one of higest cost component in the shallot farming (Nurasa and Darwis 2007). In the end the cost of shallot production to be increased which affects the retail price. Shallots price have a significant effect to the production of shallot (Paranata and Umam, 2015), it because when the shallot price increase, it will increase seed price which ultimately will increase inputs price.

Per capita consumption of shallots in 2014 was 2.49 kg/capita/year, an increase of 22.06% compared to the year 2013 is 2.07%, an estimated consumption of shallots will increase along

with the growth of the food industry, catering, hospitality, tourism and others whichused shallot as staple seasoning. Thus the supply arrangements through the organization of production, distribution and marketing of shallots in the country is important and needs to be regulated in such a way to avoid excess or shortage of supply of shallot. Thus shallot prices in the retail market are relatively more stable, not too high that would harm consumers and not until the fall that will ultimately be detrimental to farmers. Currently shallot still faces distribution problems caused by the imbalance of supply of centers of production and consumption centers. The imbalance causes the price disparity eventually rises in the cost in other areas.

4. Conclusion

The implication of the imports regulations of horticultural products, especially on shallot made of imported shallots declining trend. These regulations has been able to increase to the national shallot production. Increasing of production effected to the supply stabilitation. Meanwhile the shallots price got higher after the implementation of horticulture import regulations.

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Characterization and Resistance to Bacterial Wilt Diseases (*Ralstonia solanacearum*) of 20 Eggplant (*Solanum melongena* L.) Genotypes

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Abstract

Development of eggplant varieties resistant to bacterial wilt disease is needed to improve the productivity and quality of the eggplant. PKHT IPB have several eggplant genotypes that have the potential to be developed. To get a diversity of information sources in assembling eggplant varieties resistant to bacterial wilt disease is necessary to characterization and test of it resistance of eggplant genotypes. Research objective to obtain information about the morphological characteristics of plant genotypes are tested and to know the response its resistance to bacterial wilt (R. solanacearum). Research was conducted at trial PKHT (Center for Tropical Horticulture Studies) Pasir Kuda. The design used was a complete randomized group design one factors with three replications. Genotype 2013-070, 2013-080, 2013-064, 2013-090 is the genotypes that are resistant to bacterial wilt disease, whereas the susceptible genotype is 2013-059, 2013-076, 2013-043, 2013-043, 2013-049, 2013-021, 2013-046. Results of testing the qualitative characters to plant growth, stem, leaves, flowers and fruit showed the diversity. The color of fruit when it harvest of 20 genotypes tested consists of white, purple, and green. Results of testing the plant on quantitative characters showed it diversity.

Key words: genotype, potential yield,, characterization, Ralstonia solanacearum, bacterial wilt disease

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the vegetable crops that can grow well in tropical countries. Eggplants contain very high water, calcium, phosphorus, potassium, fiber, fulat acid, sodium, vitamins, and vitamin C (Choundhary and Gaur, 2009).

The development of Indonesia's population increase the consumption of vegetables, include eggplant. According to data from FAO (2012), the production of Indonesia eggplant occupied the sixth position in the world with a production value of 518 827 tons, lower than China, which reached production of 28.800.000 tons and India 12.200.000 tons. The low number of production can be caused by a decrease in area planted and low productivity, so that Indonesia needs to improve crop production nationwide.

Eggplants plants can be grown all year in Indonesia. Eggplants production problems in tropical countries such as Indonesia are highly susceptible to pests and diseases from seedling to harvesting stage (Raghuraman, 2008). Disease is one of the main causes losses in the production of Eggplants. The important disease in eggplant is a bacterial wilt caused by *Ralstonia solanacearum* (E.F. Smith). The disease can reduce the production from 15 to 95% (Mahmud, 1985).

The bacterial wilt are difficult to control, because the *R. solanacearum* is a bacteria that highly destructive and have a wide range of host on crop plants (tomatoes, potatoes, peppers, peanuts, papaya, etc.), ornamental plants, and weeds in the tropics and subtropics. This bacterium is a soil-borne pathogens and can survive on alternative hosts (Abdullah and Rahman, 1998). The survival of the bacteria in soil are high, especially on lands planted continuously with the susceptible host

The development of eggplant that is resistant to bacterial wilt disease is needed to improve the productivity and quality. This research was conducted on several numbers of eggplant explorations to determine the level of bacterial wilt plant resistance.

2. Material and Methods

This research was conducted at PKHT (Center for Tropical Horticulture) Pasir Kuda field in January to July 2016. This research used eggplant genotypes of 2013-008, 2013-011, 2013-012, 2013-021, 2013-023, 2013-042, 2013-043, 2013-045, 2013-046, 2013-049 , 2013-050, 2013-053, 2013-059, 2013-060, 2013-064, 2013-070, 2013-076, 2013-079, 2013-080, 2013-090. The experimental design were used by completely randomized design (RKLT) with 20 numbers of eggplant genotypes, three repetitions, and each unit consisted of 20 plants. The data were analyzed by ANOVA using SAS 9.1 software and when the data showed the real effect then continued by advance test DMRT at 5% level, and to test the correlation between quantitative characters using STAR software.

Morphological characters are classified based on the qualitative character. The characters are observed as growth habit, sinuation of margin leaf blade, tip angle, blistering of leaft blade, intensity of green color, number, size and color of flower, general shape of fruit, main color of skin at harvest maturity fruit, color of flesh, stripes of fruit, prominence of stripes fruit, intensity of anthocyanin coloration underneath calyx, size of calyx, spininess of calyx, and creasing of calyx.

Bacterial infections were observed by sampling symptomatic of plants wilt. Observations of bacterial infection carried by isolating the bacteria. Isolation was conducted by Wang (1998). Base of stems were washed with sterile distilled water. Stem cut with sterile knife, and then put in a plastic cup which contains as much as 5 mL sterile distilled water and allowed five to ten minutes. The cuts will be excreting a fluid called oose. Oose can be used to differentiate between the plant that infected bacterial wilt with fungi wilt and wither due to physiological disorders. The incidence of disease was observed starting from one week until eight weeks after planting. The formula of wilt disease incidence is using by Wang (1998).

$$P = n / N \times 100\%$$

P = Disease incidence
N = Number of plants observed
n = Number of infected plants

The level of resistance of every tested genotype based on the percentage incidence of the disease (Maharijaya, 2008), that is:
S = Susceptible (disease incidence > 75%)
MS = Moderately Susceptible (50% < disease incidence ≤75%)
R = Resistant (disease incidence ≤25%)
MR = Moderately Resistant (25% < disease incidence ≤ 50%)

3. Result and Discussion

Morphological Characters

Morphological characterization results showed 18 genotypes successfully characterized. The diversity of eggplant apparent, especially the characters on the fruit. The diversity of dominant color of skin at harvest maturity are green, white and purple. Color of Flesh is greenish and whitish. The general shape of fruit is ovoid, globular and cylindrical. Based on the results of cluster analysis, 18 genotypes of eggplant can be grouped into 3 major groups (Figure 1). The genotypes that are in one group might have had a close genetic distance as they have the same character. Differences in groups showed genetic distance over which a much. According to Singh et al. (2008) different genotypes groups with genetic distance over which a much can be utilized in breeding programs to obtain the enormous variation in segregating generations.

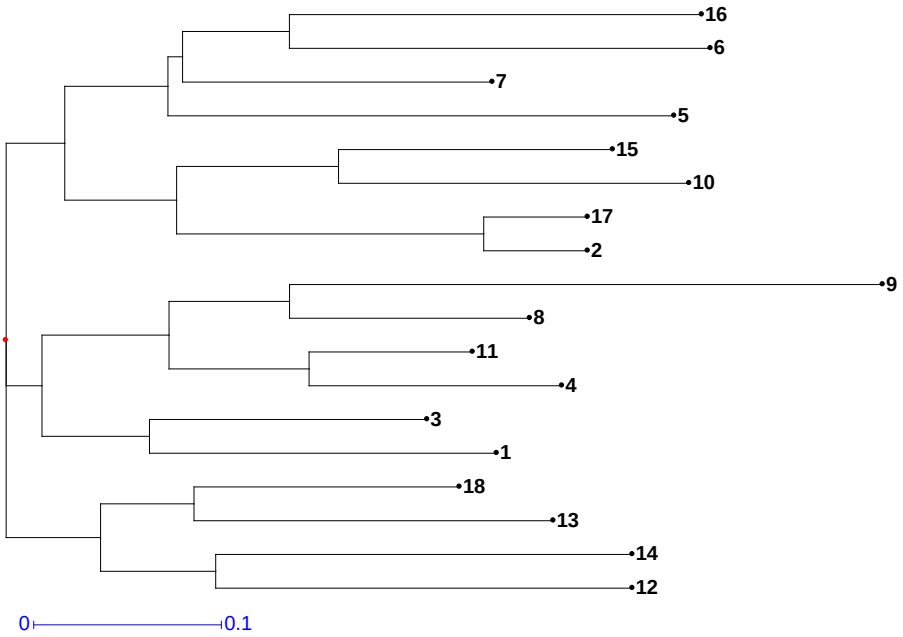

Figure 1. Dendogram based on morphological characters. 2013-008 (1), 2013-011 (2), 2013-012 (3), 2013-023 (4), 2013-042 (5), 2013-043 (6), 2013-045 (7), 2013-049 (8), 2013-050 (9), 2013-053 (10), 013-059 (11), 2013-060 (12), 2013-064 (13), 2013-070 (14), 2013-076 (15), 2013-079 (16), 2013-080 (17), 2013-090 (18).

Disease Incidence

Symptoms of *R.solanacearum* can be seen on three weeks after planting. The symptoms of this disease are leaves wilt (Figure 2a), then the leaves become yellow to brownish (Figure 2b). Heavy attack causes all part of plants be wilt, finally tip of plant be broken (Figure 2c). Symptoms of bacterial wilt disease increases as age of the plant. The disease incidence indicates numbers of eggplant infected in the population were observed. The percentage incidence of disease determines the level of plant resistance to a disease.

Figure 2. Symptoms of R.solanacearum attack. a. leaves wilt; b. leaves become yellow to brownish and c. all part of plants be wilt, finally tip of plant be broken

The infected plants in our study would die within a week or two weeks after infestation. It might be caused of the fact that our plants were infected *R. Solanacearum* at early vegetative stage. The susceptible genotypes was 2013-021 with disease incidence 73,33%. The plant would die because of the clogging xylem by *R. Solanacearum* bacterial mass, so that water transport and minerals were inhibited (Saile *et al.* 1997). The life cycle begins when *R. solanacearu*m pathogen infection entry into the roots, either through seed, water, soil and the wounding caused by insects, nematodes or agricultural tools. Upon the *R. Solanacearum* entry into the roots, the bacteria will multiply in timber vessels (xylem) in the root and stem then attack throughout the plant.

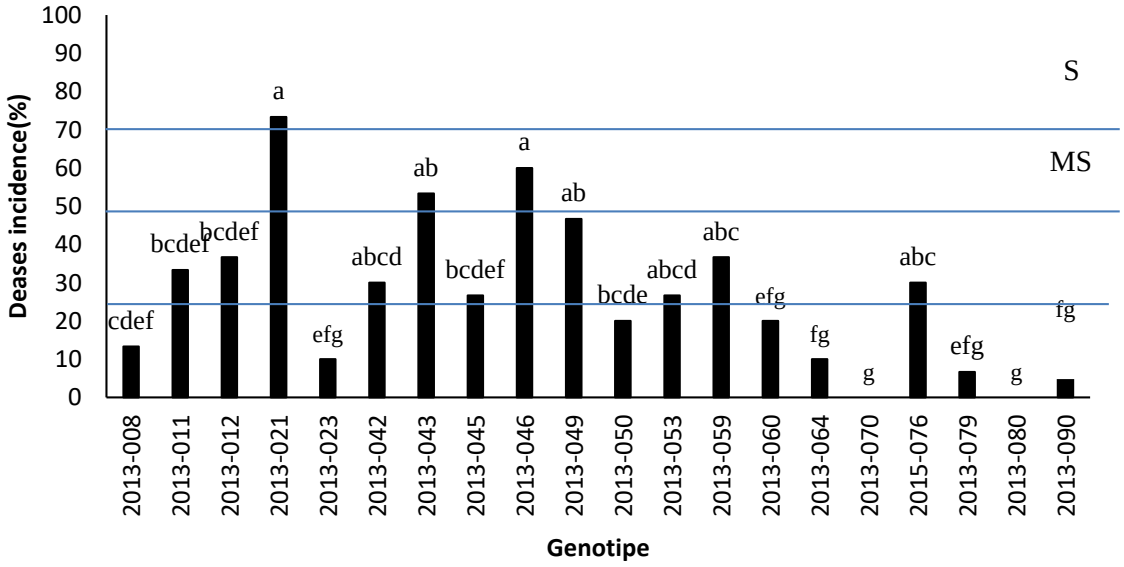

Figure 3. The percentage of disease incidence of 20 genotypes eggplant on 5 weeks after planting. S = Susceptible (disease incidence> 75%), MS = Moderately Susceptible (50% < disease incidence ≤75%), R = Resistant (disease incidence ≤25%), MR = Moderately Resistant (25% < disease incidence ≤ 50%) (Mahariiava et al. 2008)

During this researched the temperature is sufficiently moist with the rainy season. It might be cause *R. Solanacearum* expanding rapidly. According to Kelman (1953) *R. Solanacearum* grow faster at a temperature of 26-27 ° C, in addition to temperature; rainfall and relative humidity also influence in this disease.

Our results indicate that there are two genotypes resistant, that is 2013-070 and 2013-080. Both genotypes resistant to bacterial wilt disease is not showing symptoms of wilt during the

observation (up to 8 weeks of observation). The resistance response on genotypes might be caused of the genetic resistance factors.

4. Conclusion

Resistance test showed that genotype 2013-021, 2013-043, 2013-046, 2013-049, 2013-059, and 2014-076 susceptible to bacterial wilt disease, whereas genotype 2013-064, 2013-070, 2013-080, and 2013-090 resistant to bacterial wilt disease. The observation of qualitative character of the growth of plants (leaves, stems, flowers, and fruits showed a diversity. The results of the eggplant genotypes analysis were divided into three major groups. The differences eggplant group show that the diversity among genotypes. Our research show that eggplant genotypes 2013-064, 2013-070, 2013-080, and 2013-090 have resistant response to bacterial wilt disease.

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Miscellaneous

Cryopreservation for Long-term Plant Germplasm Storage

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Abstract

Germplasm is a source of genetic diversity that is essential for sustained through the long term. Seeds germplasms storage that are not resistant to desiccation can be carried out by cryopreservation. Cryopreservation is a method of storing the plant material in liquid nitrogen (-196oC) so the metabolic processes in cells, tissues or organs that are stored can be stopped so the plant material can be stored in a very long time without a genetic change or somaclonal variation. Cryoprotectants in cryopreservation storage is needed for plant material protection to adapt to the very low temperature. Some examples of successful treated-plant for cryopreservation are cocoa and papaya. In cocoa, proembryo somatic derived from cryopreservation cocoa zygotic embryo can creating embryos survived and were able to grow a callus back as much as 46.67% is dimethylsulfoxide (DMSO) and glycerol with a concentration of 15% each in MS0 solution, at a temperature of thawing (melting) 35oC by using culture media such as media restorer MS + picloram 0.1 g L-1 + thiamin 0.2 g L-1 + IAA 1 g L-1 + kinetin 0.1 g L-1. In Sukma papaya seed varieties treatment with cryoprotectants of PVS2 submersion for 30 minutes and the rate of 11-13% water content provides the best germination value (DB), the maximum growth potential (PTM), and the better rate of growth (KCT), 38.39%, 38.39%, and 2:24%, respectively.

Keywords: cryopreservation, cryoprotectant, germplasm

1. Introduction

Indonesia has a big biodiversity, including genetic diversity of plants, of which approximately 11% of the world's plant species are tucked away in the woods as a wild species and other agricultural lands spread over thousands of islands in Indonesia (Leunufna 2007). Sources of genetic diversity of plant crop as germplasm is very important to be utilized in the future, for the science development and understand in depth the plant's benefits. Germplasm is a term of living organism or groups that determine their characteristics (Mugnozza and Perrino 2002).

Along with land exploration due to population race, uncontrolled forest exploitation, as well as the growing number of high-yielding varieties of plant breeding, it can create the wealth of plants available plasmanutfah shrinking very rapidly. Wattimena and Ansori (1992) states that the collection and preservation of plasmanutfah needed both to improve the quality and quantity of plants and is also required to facilitate the testing of other experiments with certain techniques. Thus the storage and preservation seed plants are not only intended to supply planting the following season, but can also be intended to supply the medium term and long term.

Germplasm preservation in plants can be carried out either in situ or ex situ outside the habitat. In situ plant preservation is an attempt to maintain the plants growth in the natural habitat. This method is benefit for plants because plants will not adapt to stress condition on the new environment, but also has some risks against the possibility of germplasm loss in open field and operating costs (Leunufna 2007), in addition to biotic and abiotic stress also affects the level of genetic diversity of plants its self (Roostika 2013). Ex situ plant preservation is a such plant conservation by moving the plants from the growth point of origin. By using ex situ preservation, the collection can be observed. Ex situ preservation includes botanical gardens, collection, storage of seeds, in vitro preservation, pollen storage and genebanks (Engelmann 2000).

The germplasm storage system are not the same from one plant to another. This is due to the diversity of the natural characteristic and condition of the crop. The diversity of the seeds character will make the way of germplasm plants storage are also not the same from one plant to another. Most plants produce orthodox seeds, where the seeds can be dried up by the resilience of the water content (KA) <7% and stored at low temperature, so the seeds do not have problems when stored for a long period of time (Engelmann 2000). Most other plants produce seeds are recalcitrant, where the seed is less resistant or have constraints to be saved in the long term because it is not resistant to low temperatures and low water content, where resistance moisture content> 20%. Seeds that have condition of orthodox seed but resistant to moisture (KA) 10-12% referred to intermediate seeds (Uyoh *et al*. 2003). Approximately 70% of tropical plant species produce recalcitrant seeds, including forest plants, crops and fruit trees (Normah 2000).

Germplasm storage solutions for plants with intermediate and recalcitrant seeds usually through in vitro culture. In connection with the storage length of in vitro techniques can be divided into two general categories: (1) storage of short or medium term with the aim of simply growth suppressing for a while, using a low temperature, the addition of inhibitors, the addition of mineral oil and impoverishment of the culture media, and (2) with the objective of long-term storage, where the metabolic activity actually stopped but the cells keep live. In this case usually used liquid nitrogen (N2) with a very low temperature (-196^{o}C). This technique is known as cryopreservation.

In general if the plant material is stored at very low temperatures can cause damage to the plant cell. The plant cell membrane will act as a barrier to the formation of ice crystals from the water content within cell so the cell can not survive if a temperature of liquid nitrogen are very low. During freezing and melting, plant cells can be damaged as a result of being forced to adapt with very low temperature, the formation of ice crystals, and dehydrated (Reinhoud *et al.* 2000).

Cell damage that occurs when plant material facing very low temperatures can be overcome by dehydration or drying stages to avoid the formation of ice crystals using cryoprotectants, such as glycerol, dimethylsulfoxide (DMSO), and sucrose. When the cryopreservation process in plants has been completed and the plant will be continously activated then cryoprotectants should be immediately removed from the planting material through washing stages with liquid culture such as cryoprotectants but it can be toxic to the plant cells (Watanabe *et al.* 1999). In general, the stages of cryopreservation techniques is including pre-growth, pre-culture, loading, dehydration, freezing, melting, replacement charge (deloading or unloading), recovery, and regeneration (Roostika 2013).

2. Methodology

In general, the cryopreservation technique is often done by classical and new cryopreservation techniques. Classical cryopreservation techniques (slow cooling cryopreservation) which cover or soaking the planting material in a solution of cryoprotectants and stages to reach the temperature that can freeze, followed by storage in liquid nitrogen. Techniques of new cryopreservation (fast freezing cryopreservation / vitrification), based on the phase transition from liquid form into the shape of non-crystalline or amorphous glassy (invisibility) for the planting material are placed on media cryoprotectants that highly concentrate then immediately placed in liquid nitrogen (Engelmann 2000). Solution in vitrification often used PVS2 [*Plant Vitrification Solution* 2 by Sakai *et al*. (1991)], which contains 30% gliserol, 15% dimethylsulfoxide (DMSO), and 15% etilen glicol (EG)].

Cryopreservation in plant cell culture and in the culture of others, includes several steps: providing sterile tissue, the addition of cryoprotectants and providing early treatment, freezing, storage, melting, viability testing and plants growth and regeneration. Increasing the viability of planting material either morphologicaly and physiologicaly must be improved because it will adapt to the freezing conditions at temperatures of -196^{o}C.

Some networks or institution often manage the planting material to be stored in the cryopreservation in form of apical meristem and lateral organs of plants (embryo, endosperm, ovule, anthers / pollen), seeds, cell culture, somatic proembryo, protoplast, callus, etc. In general, the smaller planting material is rich in cytoplasmic and meristem cells will be able to last longer in this cryopreservation technique.

Permeability of cells during cryopreservation needs to be maintained by adding protective material in the form of a solution called cryoprotectants. The application of cryoprotectants will keep the form of the cells during the freezing process occurs by increasing the concentration of the solution and prevent the formation of ice crystals by dehydration or drying stage (Simione 1998). Cryoprotectants are frequently used and give the best effectiveness are dimethylsulfoxide (DMSO) and glycerol. Cryoprotectants should be used in the appropriate composition and soaking time.

3. Discussion

Some plants, especially plants that have recalcitrant or intermediate seed properties has been successfully stored in cryopreservation. Examples of successful treated-plant for cryopreservation are cocoa and papaya. In cocoa, proembryo somatic derived from cryopreservation cocoa zygotic embryo used as an plant material of slow cooling technique, because proembryo somatic cell more compact, has denser cytoplasm and still in the globular phase. The combination of cryoprotectants that creating embryos survived and were able to grow a callus back as much as 46.67% is dimethylsulfoxide (DMSO) and glycerol with a concentration of 15% each in MS0 solution, at a temperature of thawing (melting) 35^{o}C by using culture media such as media restorer MS + picloram 0.1 g L-1 + thiamin 0.2 g L-1 + IAA 1 g L-1 + kinetin 0.1 g L-1, as shown in Figure 1 (Hervani 2006).

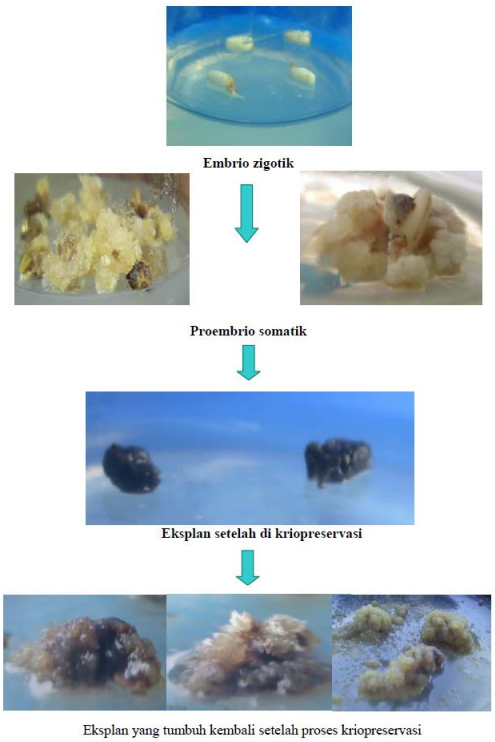

Figure 1. Development of proembryo somatic explants after cryopreservation using cryopreservation method of slow cooling at the age of 24 weeks after planting

In the next stage of the study, the researchers work with Sukma papaya seed varieties whose seeds are intermediates seed to be preserve in the long term as cryopreservation. Treatment with cryoprotectants of PVS2 submersion for 30 minutes and the rate of 11-13% water content provides the best germination value (DB), the maximum growth potential (PTM), and the better rate of growth (KCT), 38.39%, 38.39%, and 2:24%, respectively. Seed without cryoprotectants is not able to grow back and the seeds are soaked too long in PVS2 deliver the low value of DB and KCT (Hervani *et al.* 2016).

Seeds that are not protected or immersed in cryoprotectant prior to entry of liquid nitrogen is not able to grow at all. This is because the cells contain a lot of water so it would not survive if a temperature of liquid nitrogen are very low. During freezing and melting process, plant cells can be damaged due to: (1) force adaptation a very low temperature, (2) the formation of ice crystals, (3) dehydrated, and (4) the formation of free radicals. During the cooling process, the lipids in the membrane will undergo a transition phase from the liquid crystal into a gel phase. This process results in leakage of the cell so the cell becomes damaged, since not all the lipid transition phase at the same temperature. At low temperatures, some proteins in plant cells will be inactive due to the nature of the protein that is sensitive to low temperatures (Reinhoud *et al*. 2000).

4. Conclusion

Cryopreservation currently offers the only safe and cost effective option for the long-term conservation of genetic resources. An important key to the activities of cryopreservation is appropriate cryoprotectant concentration, soaking period time, the selection of planting material to be stored in cryopreservation, thawing process and reactivation cell growth after cryopreservation.

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Good Manufacturing Practices (GMP) for Fresh-Cut Fruits and Vegetables

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Abstract

Fresh-cut fruits and vegetables industry has been growing rapidly in the last decade. Its practicality and fresh look is convenient and fitting for the current lifestyle and consumers' preference of high quality products. Fresh-cut produce is very perishable due to the physical stress it had been subjected to, i.e. peeling, cutting, slicing, and shredding. Thus, fresh-cut produce may have a shorter shelf-life compared to the intact produce. Considering the importance of food safety and costumers' higher awareness of it, fresh-cut industry unavoidably should implement Good Manufacturing Practices (GMP) in their factory. Good Manufacturing Practices is a system consisted of procedures that had been learnt from experience and verified by scientific testing. GMP is highly important not only for food safety reason but also because it helps agribusiness beginners to produce high quality products. Most countries in the world have their own GMP certification system for agricultural products. Indonesia has one issued by Minister of Agriculture which includes the good practices guidelines for facilities and infrastructures, processing, storage, work security and safety and environment management, workers health and hygiene, monitoring, recording and tracebacking, certification and coaching. GMP for fresh-cut produce may include the quality assurance of raw materials, sanitizing, packaging, and monitoring of microbial contaminations. These stages in the handling of fruit and vegetable are the very determining stages for the quality of fresh-cut produce.

Keywords: Food Safety, Fresh-cut, Fruit, Good Manufacturing Practices, Vegetable

1. Introduction

Fresh-cut fruits and vegetables industry has been growing rapidly in the last decade. The increasing awareness of healthy dietary habit leads people to consume more fresh fruit and vegetable. The practicality and fresh look of fresh-cut produce certainly is convenient and fitting for the current lifestyle and consumers' preference of high quality products. Initially, fresh-cut produce was only served by street vendors and restaurants, who simply wash, cut and serve the fruits and vegetables to their consumers. Recently, fresh-cut produce are available in supermarkets and packed in fancy packaging. Fresh-cut products are now marketed not only in the form of ready-to-eat fruits and vegetables but also ready-to-cook or meal substitution (eg. mixed with noodle, bread, shrimp, egg, chicken, etc).

Fresh-cut produce are very perishable due to the physical stress it had been subjected to, i.e. peeling, cutting, slicing, and shredding. Thus, fresh-cut produce may have a shorter shelf-life compared to the intact produce. It has realatively fast quality deterioration, such as loss of texture, brown stains, off-odor and decay. Fresh-cut produce commonly experiences high

respiration rate, the extra handling steps may cause wounding, cutting and loss of microbial barriers. Therefore, the fresh-cut industry is challenged to process and distribute quality products that are considered fresh and safe (Kim, 2016).

These problems require a quality assurance system in order to enable agribusiness owners to manufacture products with constant, safe and uniform quality. Good Manufacturing Practices is a system consisted of procedures that had been learnt from experience and verified by scientific testing. GMP is highly important not only for food safety reason but also because it helps agribusiness beginners to produce high quality products. Moreover, consumers awareness on food safety is increasing, thus GMP compliance can be used as a powerful product differentiator and marketing message (Anonymous, 2015; Liu, 2007).

2. Technology requirement for fresh-cut fruits and vegetables

a. Raw materials

Being very perishable and vulnerable, it is obvious that high quality raw materials are required to produce high quality fresh-cut fruits and vegetables. The quality of fresh-cut products mainly depends of the intact fruit and vegetables, thus good practice from farming to harvesting, followed by good handling and preparation, is a must. Leafy vegetables senesce rapidly compared to fruit vegetables and root vegetables. Fresh-cut processing of leafy vegetables may have unique postharvest problems due to the detachment of leaf from the whole plant. Therefore, leafy vegetable used for fresh-cut produce has to be handled with more postharvest care compared to other intact vegetables which are used for fresh-cut produce (Kim, 2016).

Quality can only be maintained after harvest, and cannot be improved (Acedo, 2016). Time of harvest is one of the determining factors of quality for fruits and vegetables. Immature state to a mature and ripe state are exhibited internally and externally. These changes can be used to help determine the optimum indicator for time of harvest. Suitable method of harvest is also needed to be carefully chosen. Hand harvesting is used for most horticultural produce. The advantage of this method is it reduces damage as opposed to mechanical harvesting. Hand harvesting allows accurate selection for maturity and quality by trained labour (Nenguwo, 2016).

Harvest at cooler time of day or harvesting under shades may lower the product heat and increase labour efficiency. It was reported that iceberg lettuce (cultivar; Sacramento) harvested in winter has less respiration rate compared to lettuce harvested in summer and fall (cultivar; U-lake) (Kim 2007b). Respiration rates of mushroom (Agaricus bisporus Sing.) harvested in hot temperature summer and autumn seasons were lower than mushrooms picked in low temperature spring and winter seasons. Summer-harvest mushrooms and mushrooms kept at 2°C for more than 7 days after harvest were unsuitable for fresh-cut processing due to rapid browning (Lim et al. 2004).

Avoid harvest during or right after rain when wet conditions increase risk of spoilage of produce. Carefully harvest using proper tools and prevent from dropping produce to the ground to avoid contamination and infectation of microbia and diseases (Acedo, 2016).

b. Postharvest technology

Postharvest begins where production ends, i.e. at harvest (Acedo, 2016). Packing activities both in field and shed packing house must also note that it is very important that the produce is handled with great care to prevent any damage, such as from dropping, bruising or rubbing.

Bruises are entryway for diseases and microbia infectation, leading to reduced shelf-life and marketability (Nenguwo, 2016).

The preparation of fresh-cut produce is then continued in distributing packing house. One of the bigger challenges of fresh-cut packaging are to determine the optimum modified atmosphere (MA) packaging material (mostly film), to maintain the convenience of the packaging and to use enviromental-friendly packaging materials (Kim, 2016).

The main activities that take place within a packing house, usually includes: washing, peeling, trimming/cutting, dehydration, packaging, inspection, and shipping. Certain produce may need different treatments, eg. potato is heat treated (30^{o}C for 24 hour) before peeling and trimming, and then followed by immersion in cold water before packaging. Iceberg lettuce needs to be cooled right after harvested and stored in cold storage before cut/trimmed. Washing is done afterwards, followed by dehydration before packaging and inspection (Kim, 2016).

Some of the major defects occured on fresh-cut produce are browning and early wilting. Various technologies had been established to delay browning of fresh-cut produce. Heat treatment (potato, onion) and cold water immersion (some leafy vegetables) are common practices (Hong *et. al.*, 2004; Kim *et. al.*, 2006; Lim *et. al.*, 2005). Natural anti-browning treatments, such as ascorbic acid, had also been reported to be effective. However, its application in industrial scale may be impractical and costly (Kim, 2016).

3. Good Manufacturing Practices (GMP)

The International Organization for Standarization had established ISO 22000 for Food Safety Management standard. Figure 1 presented the steps for an agribusiness practitioner to be ISO 22000 certified. HACCP (Hazard Analysis and Critical Control Points) principles collaborated with management systems elements had to be implemented first. For HACCP system to function well and effective, it needs to start with the fulfillment of pre-requisite program, which is necessary as the basis of environment condition and activities that are held in a food industry. Good Manufacturing Practices) (GMP) has the role in assurance of food safety and is align with the pre-requisite of HACCP implementation. The description of HACCP pre-requisite is very familiar with that of GMP, i.e. concerning sanitation and hygiene of food preparation and processing. GMP widely focuses on operational aspects of activities involved in the factory and personnel operation (Anonymous, 2013).

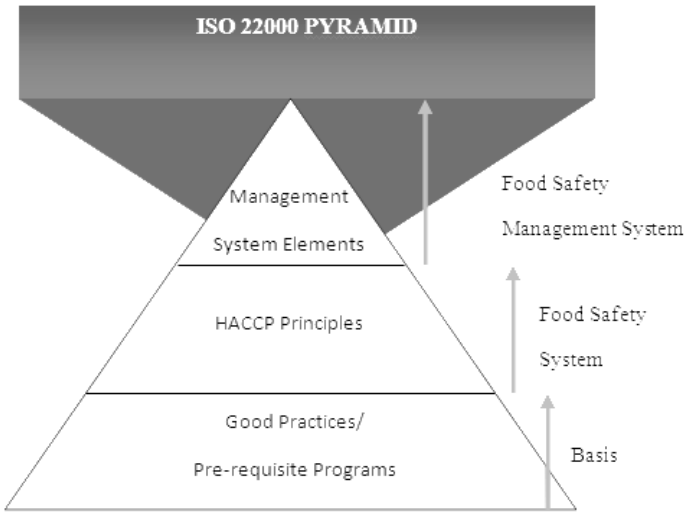

Figure 1. The international standarization in agriculture business. (Source: www.globalcertfoodsafety.us)

GMP is one of the main pre-requisite programs before a food industry can be certified for HACCP system (Harris, 1999). Agriculture business producing food products will generally need to apply several good practices in their system (Figure 2). With these good practices combined, the agribusiness owners will not only entitled to HACCP certification but also will be ready for their ISO 22000 certification, provided that they have their management system elements ready.

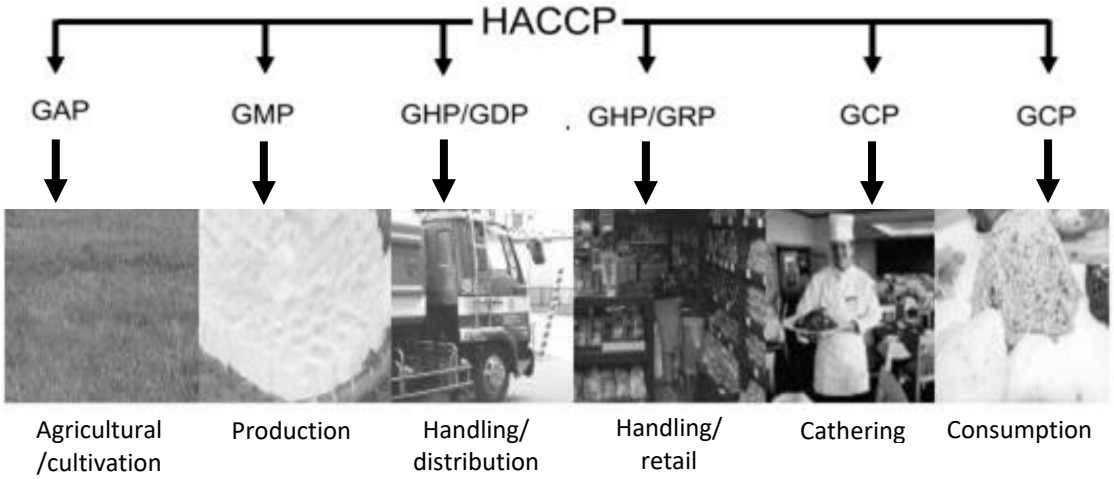

Figure 2. The good practices as pre-requisite programs for HACCP implementation (Source: Raspor, 2008).

GMP is known as a system to ensure that products meet food safety, quality and legal requirements. GMP contains explanations and illustrations of the minimum requirements and general practices of food handling in all aspects of food processing, from raw material to end products. It is basically composed from procedures learnt from experience and verified by scientific testing, thus it is science based. It is constantly being improved, cost-effective and ensures the production of safe high quality products (Anonymous, 2015).

There are three main reasons for implementation of GMP in a food production unit. The first main reason is to ensure food safety. As mentioned above, GMP is an important part of overall HACCP food safety system in a food business. It enables a food business to produce food safely (Liu, 2007). GMP is required in all relevant food legislation and customer certification standards. Moreover, it helps the business management to develop a good quality assurance system.

GMP is very important for agribusiness owners, especially beginners. It provides basic principal in spesific food production. GMP leads agribusiness practitioners to fulfill the pre-requisites of productions in handling their products, from raw material to ready to consume products. That way, GMP helps them producing high quality products, safe for consumption, according consumers demands. With the high quality of their products, the competitiveness is also improved. Increased productivity and efficiency of processing activities brought by GMP implementation will also be a desirable benefit for the business (Anonymous, 2015).

GMP is also important for marketing advantages reason. Food marketing is an always growing business. Food industries are required to comply with GMP regulations, and with the consumers awareness increasing, nowadays more and more food industries comply (Liu, 2007). Products safety information is easily widespread, and consumers are voluntarily spread both the positive and the negative news on food safety. Local supermarket chains and retails

has started to ask for extra quality requirements including food safety certification. Thus, GMP compliance can be used as a powerful product differentiator and marketing message.

4. GMP for fresh-cut fruits and vegetables

Generally, in establishing a GMP guidelines, General Principles of Food Hygiene developed by Codex Alimentarius (1969) is referred to. It is considered to cover all stages along the food chain. Details of the principles include: primary production, establishment (design and facilities), control of operation, establishment (maintenance and sanitation), establishment (personal hygiene), transportation, product information and consumer awareness, and training (Tippayawat, 2016).

Most countries have their own institutions or bodies who will issue and establish a certificate of GMP and HACCP for food production. Every country should also have their own regulations about the standardization for food quality. In Indonesia, the regulation is issued by the Minister of Agriculture. Regulation of Minister of Agriculture on requirements and Implementation of Good Manufacturing Practice for Agricultural Products No. 35/Permentan/OT.140/7/2008 (MoA, 2008) was issued and effective since 2008. The regulation's scope included (1) facilities and infrastructures, (2) processing, (3) storage, (4) work security and safety and environment management, (5) workers health and hygiene, (6) monitoring, recording and tracebacking,(7) certification and (8) coaching.

Fresh produce may have chemical and microbiological risks. To prevent the risk of chemical residues, eg. pesticide, raw material specifications should be established in order to ensure proper selection and sorting of raw materials from suppliers or preferably from GAP implemented or certified farms. Testing of raw material may be considered necessary as additional safety management (Tippayawat, 2016).

Chemical risk may also exist in the activities held within the packing house, eg. washing produce using sanitizers. The most popular sanitation for fresh-cut produce is chlorine. It is widely used to reduce microbial contamination. Proper rinsing should be done thoroughly in order to wash away the chlorine from the fresh produce. However, there is growing interest in developing a safer and more effective antimicrobial alternatives to chlorine, such as electrolyzed water and ozonated water (Kim, 2016).

Microbiological contaminations may occur both at farms and packing houses. As previously mentioned, dropping produce or bruising may be the entryway of contaminations. Some produce may look uninjured in appearance, while actually it was contaminated and the disease grows from within the fruits.

The location and the design of lay out and processing utilities is highly important in preventing microbiological contaminations. The agribusiness owner should ensure that the location is properly located and free of any possible contamination from its environment. The building should be built with concern to technical and hygiene requirements, and with considerations about layout, ventilation, lighting, etc. It is important to design the floor plant based on the production method, as it will also affect the layout of machines, location of ventilation, lighting, drainage, etc. Environment management and monitoring includes waste management, pollution control (transportation, hot steam, smoke, noise, etc). Monitoring of the effectiveness of environment management facilities and environment quality should be done internally, for example on the processing and reutilization of waste (MoA, 2008).

Frequently utilized tools such as knives, slicers, cutting boards and conveyors are among the most contaminated tools in packing houses (Seo *et. al.*, 2007). Fresh-cut produce are also

at risk of cross-contamination from environment and workers. Workers hygiene (hygiene facilities and personal hygiene) is a basic requirement in GMP implementation. It is prohibited for workers to enter and work at the plant site if he/she has respiratory infection, gastrointestinal infection, and skin infection. Self hygiene must be taught to all workers and be practiced at all time in the production process. Workers should wash their hands thoroughly before and after processing, wear certain personal apparel (eg. face mask, hand gloves, hair cover, etc) and no jewelry, never eat and drink at certain places, and all areas of the production site should be prohibited for smoking. Workers commitment will be necessary in the implementation of this SOP (Bawalan and Chapman, 2006).

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Breeding of Anthurium (*Anthurium andreanum*) : A strategy to produce new clones as tropical ornamental plants

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Abstract

Anthurium is a tropical ornamental plants which is have a high value in floriculture industries.Hibridisation is one strategy to create new clones in anthurium. The objectives of these research were to find out of time ripening fruits in anthurium and percentage of seedling from hibridisation which is succesfully to produce seed. The research was conducted in Indonesian Ornamental Crop Research Institute (IOCRI). Collection of anthurium germplasm in IOCRI were used as breeding materials. Hibridisation was conducted at 7 to 9 am. The observation parameters were morphology characters, time of ripening fruits, percentage of seedling and total number of seed every fruit. Fruits were produce 4 to 9 months after hibridisation. Percentage of seedlings were 58,33% to 100 % and average of total number of anthurium per fruits were 21 to 110 seeds. There were some variations in morphology characters in F1 population.

Keywords : *Anthurium andreanum*, hibridisation, new clones, morphology character.

1. Introduction

Anthurium andreanum is one of potential tropical ornamental plant, which is have a high value in floriculture industries. Genus of anthurium is devided into 1500 species, and the biggest cluster in *Aracea* family. Anthurium is native from Colombia, and distributed from London to Hawaii in 1889 by S.M Damon (Higaki *et al.* 1995). Habitat of anthurium is tropical forest, wet forest and high land until 1500 m. Anthurium is growing as epifit plant, terestial or epipetrik in dry area as west Mexico (Croat. 1986).

Anthurium have specific characteristic, there are spathe and spadix (Chen et al. 2011; Gopaulchan et al. 2013). Anthurium was produced as cutting flower, potted flowers and landscape (Nowbuth et al. 2005). Anthurium was increased in market's demand in 2007 and there was in 9th International market ranked. "Anthura" The Netherland is the biggest produsen and exporter of anthurium in the world. The second produsen is "Mauritius" Hawaii. The market of "Anthura" was Europe and market of "Mauritius" is United State (Islam *et al.* 2013).

Anthurium market always change based on consument preference, quality product and market demand. The preference like as mode, changes everytime particularly the novelty product. One of strategy to achieve some novelties anthuriums are breeding of anthurium to create new clones and varieties. Some strategies of breeding were conducted by some methods such as biotechnology, mutation breeding and conventional breeding. This research is one of stategy in breeding using conventional hibridisation method.

Currently, *Anthurium andreanum* was hibridisation product among species anthurium *andreanum, Anthurium antioquiense, Anthurium ornatum, A.andreanum* and *A. ornatum*, there

are diploid 2n = 30 (Sudarshini *et al.* 2014). Breeding of anthurium can enhance the genetic variability and create new varieties, which is make gene pool of anthurium increasing. The most important of breeding is pollination. Some insects were needed in pollination by naturally (Kraemer and Schmitt, 1999). Naturally pollination was produced random of genetic variability, that why specific character couldn't achieve directly. The specific character can be achieve by hibridisation based on human breeding programmes. The specific characters which is become breeding purpose were improvement of quality product as shape of flower, color and size of flower (Rosario and Aurique. 2009),vase life (14- 28 days) (Elibox and Umaharan. 2008) and resistance to blight caused *Xanthomonas camprestris* (Kuehnle *et al.* 2004).

The pollination was conducted on 7-9 a.m, when pollen was receptive and mature. Beside the time, successfully of breeding was depend on selection of anthurium parental, compability and breeding environment such as fertilizer, drainase, lighting and humidity (Chang *et al.* 2010). The limited of breeding in anthurium was differently of maturing male and female pollen.

The objectives of the research were to find out of 1) Time ripening fruits in anthurium, 2) Percentage of seedling from hibridisation which is succesfully to produce seed. The observation datas were used for prebreeding recommendation to create new clones and varieties.

2. Materials and Methods

Breeding of anthurium was conducted on April 2014- December 2015, in Indonesian Ornamental Crop Research Institute (IOCRI) Segunung. Collection and germ plasm of anthuriums in IOCRI were used as materials breeding. There are some cultivars of anthurium such as anthurium cv. Alvin, Lady jean, Tivoli, Saxo, Farran, Castano, Safari etc). Pollination were conducted in the morning (7-9 a.m). Some parentals have characters as breeding purpose such as color, shape and size of flowers, potted plants or cutting plants. Color character is one of purpose in breeding programme of anthurium (Table 1).

Table 1.Color of anthurium cultivars as parental in breeding programme.

No.	Female	Male
1.	Anthurium Alvin (white-pink, big size)	Anthurium Tivoli (dark red)
2.	Anthurium Saxo (greenish red)	Anthurium Bonito (purple, small size)
3.	Anthurium Bonito (purple, small spathe)	Anthurium Saxo (greenish red)
4.	Anthurium Farran (red, big spathe)	Anthurium Holland Putih (white)
5.	Anthurium Safari (maroon, stripped spathe)	Anthurium Castano (red)
6.	Anthurium Octavia (red, greenish spot)	Anthurium Alvin (pink-white)
7.	Anthurium White Champion (white, big spathe)	Anthurium Boneto (purple, small spathe)
8.	Anthurium Violeta (violet, big spathe)	Anthurium Sempre (greenish red)
9.	Anthurium Boneto (purple, small spathe).	Anthurium Qtazu (dark red)
10.	Anthurium Lady Jean Pink (big spathe, pink)	Anthurium Alvin (pink-white)
11.	Anthurium Boneto (purple, small)	Anthurium Fesca (red)
12.	Anthurium Max Farde (red, big spathe)	Anthurium Holland Putih (white)

The breeding characters as purposes in this research were color of spathe anthurium (maroon, red,purple, green), short internode and more than 4 inflorescence. Conventional breeding as one of strategy consisted 4 steps:1) Crossing and hibridisation, 2) Harvesting, 3) Sowing anthurium seed and 4) Evaluation dan selection.

a. Crossing and Hibridisation

Crossing and hibridisation were conducted in the morning (7-9 a.m). The male mature pollen was put in female spadix. The resiprocal crossing was conducted when pollen enough.

b. Harvesting

Pollination was successfully in crossing if there were some swelling in spadix and formation seed pod. The yellowing or browning pod harvested in several months after crossing.

c. Sowing anthurium seed

Seeds were sow in charcoal medium and germination in 2 weeks. Germination was replanting in bigger chamber pot (pot diametre : 10 cm; 15 cm and 20 cm).

d. Evaluation and Selection

There were two kinds of selection, vegetative and generative selection. Vegetative selection was carried out to select of vigorous germination and vigorous plant before they were blooming. The observation parameters were plant height and total number of leaves. Generative selection was find out the variability of flower and comparing with their parental in F1 population, color of spathe, shape and size of flower (qualitative characters).

Observation Parameters

In this research, the observation parameters were 1) Morphology characters, 2) Time of ripening fruits, 3) Percentage of seedling and 4) Total number of seed every pod.

1) Morphology character consisted color, size and shape of spathe and spadix. Observation used RHS color chart for color and UPOV guide characteristic of anthurium for size and shape of spathe and spadix.
2) Time of ripening or pod were observed from start of crossing time until formation pod seed and pod harvested
3) Percentage of seedling were counted total number of germination seed times 100% and devide this result with total number of seed in one pod.
4) Total number of seed every pod. Counting how many seed in one pod.

Stastistical Analysis

Statistical analysis were performed using SPSS software.

3. Result and Discussion

Hibridisation of anthurium were carried out to increasing genetic variability and create new clones and varieties as tropical ornamental plants.Varians were formed by crossing parental as new gene resources and release as new varieties. All of varians were depended on their parental, both female and male parents. Formation of seeds or pods were 4- 9 months after crossing (Table 2).

Table 2. Time of ripening pod of anthurium (month after crossing)

Female	Male	Pod formation and pod ripening (months)
Anthurium cv. Alvin	Anthurium cv. Sempre	4
Anthurium cv. Alvin	Anthurium cv. Holland Putih	4
Anthurium cv.Arch.Hawaii	Anthurium cv Flex	4
Anthurium cv.Tivoli	Anthurium cv.Sempre	5
Anthurium cv.Violeta	Anthurium cv.Alvin	7
Anthurium cv. Mutiara	Anthurium cv. Flex	9
Anthurium cv.Arch.Hawaii	Anthurium cv.Castano	4
Anthurium cv. Saxo	Anthurium cv. Sempre	5
Anthurium cv.President Pink	Anthurium cv. Castano	5

In color character, anthurium cv. Alvin have pink- white color and big spathe (Fig.1). In this research, the reciprocal crossing can not conduct it. The reason is limited of pollen and different time of pollen maturing. Female pollen of anthurium were maturing first than male pollen.

Pod formation were different, depend on parental characters, genetic, and time of pod formation. The faster of pod formation was 4 months after crossing and the longest was 9 months. The anthurium Arch.Hawaii dan Alvin as parental were produced pod earlier. Both of cultivars as female parental (pod formation in 4 months) (Table 2). It means that parental have easy and faster to pod formation character.

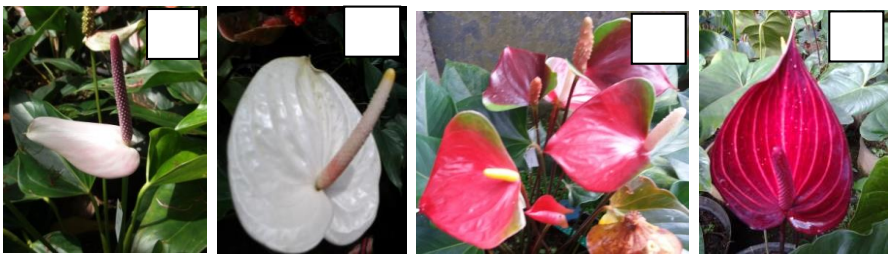

Figure 1. Anthurium parental. Anthurium cv.Alvin (A), Anthurium cv. Holland Putih (B), Anthurium cv. Sempre (C), Anthurium cv. Safari (D).

In this experiment and crossing, 100% crossing were successfully to produce seed (Fig.1). One of all germplasm, anthurium cv. Safari were not produce seed pod.

Fig 2. Seed of anthurium . Harvesting pod (A, B and C).

All of seed (almost 100%), there were 100 % survival germination, but anthurium cv. Tivoli x Sempre, just 58,33% germinate (Table 3).

Table 3. Total number of seed and percentage of survival germination.

Crossing	Total number of seed	Survival of germination (%)
Anthurium cv. Alvin x Anthurium cv. Sempre	21	100
Anthurium cv. Alvin x Anthurium cv. Holland Putih	60	100
Anthurium cv. Arch.Hawaii x Anthurium cv Flex	110	100
Anthurium cv.Tivoli x Anthurium cv.Sempre	70	58,33
Anthurium cv.Violeta x Anthurium cv.Alvin	42	100
Anthurium cv. Mutiara x Anthurium cv. Flex	31	100
Anthurium cv. Arch.Hawaii x Anthurium cv.Castano	83	96,39
Anthurium cv. Saxo x Anthurium cv. Sempre	30	100
Anthurium cv. President Pink x Anthurium cv. Castano	70	100

The survival of germination were depend on seed maturing and pod time harvesting, post sowing of seed, and environment (light, temperature, humidity, fertilizer and drainase). Crossing of anthurium cv. Tivoli x anthurium cv. Sempre showed the lowest germination. The reason was broken seed from fungus contamination when sowing of seed. High of humidity enhanced of fungus survive and bad effect in germination.

4. Conclusion

- Genetic character of parental is one of the essential elements which is influence in anthurium breeding to create new clones through conventional breeding. Anthurium cv. Alvin as female parental was produced fastest pod formation.
- Seed and pod formation achieved 4 months after crossing.
- 100% germination were succesfully produce survival seedling.

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