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"Improving Food Security : The Challenges for Enhancing Resilience to Climate Change"

Volume I

The University of Lampung

Indonesian SEARCA Fellow Association

Southeast Asian Regional Center for Graduate Study and Research in Agriculture

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Improving Food Security : The Challenges for Enhancing Resilience to Climate Change

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SEARCA

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Preface
COMMITTEE CHAIR

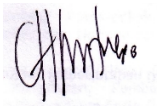
Recently, there are many discussions about food security as a complex issue of sustainable development. One of important topics is will the food needs in the future be met by the current production levels? In addition, the future production faces another sustainable development issues, one of which climate change that affects all four food security dimensions: food availability, food accessibility, food utilization and food systems stability. Improving food security, therefore whilst reconciling demands on the environment conditions which becoming the greatest challenges.

To response that challenges, The University of Lampung collaborated with ISFA (Indonesia SEARCA Fellow Association) and SEAMEO-SEARCA conduct an International Seminar on "Improving Food Security: The Challenges for Enhancing Resilience to Climate Change" in Bandar Lampung, Indonesia on August 23-24, 2016. There are 4 topics are offered as follows: (1.) Food Security and Food Production System, (2.) Food Security, Post Harvest Science and Technology, (3.) Food Security and Socio-Economic Environment Aspect and (4.) Ecological Perspectives on Food Security.

At this seminar, 111 research articles were submitted from 6 countries i.e. Indonesia, Lao, Malaysia, Myamar, Thailand, and Vietnam. The authors are researchers, practitioners included NGO, policy makers, academics as well as industrial professionals. The ultimate aim of this seminar is to deliver state-of-the-art analysis, inspiring visions and innovative methods arising from research in a wide range of disciplines. Through this activity, it is expected that research articles in all aspects related to food security can be documented, rapidly spread, communicated and discussed throughout the countries.

Thank you for your participation and looking forward to having productive discussion among participants.

Sincerely yours,



Christine Wulandari, Ph.D

Preface
The University of Lampung Rector

Many Asian countries face serious challenges on their food security due to changing consumption patterns including the demographics, declining of agriculture productivity, degradation of natural resources, rising input costs as well as cost for transportation of supply chains. All of these, need various trends anticipation of short to medium term, and this is clearly becomes efforts focused on mitigating towards the challenges. Together with SEAMEO-SEARCA and Indonesian Searca Fellows Association (ISFA), the University of Lampung (Unila) collaborated to conduct an international seminar with theme in "Improving Food Security: The Challenges for Enhancing Resilience to Climate Change" on 23-24 August 2016 in Emersia Hotel, Bandarlampung. From this international seminar, 111 research articles from six countries in Southeast Asia were compiled and expected to be used as a stepping stone for preparation of development strategies in Indonesia country or other Asian countries resolving the issues of Food Security.

This cooperation among Unila with ISFA and SEARCA in accordance with the Unila statement mission for Unila goals of 2005-2025, one of which Unila is able to build joint effort in many development aspects within various parties, including governments, publics, businesses, non-governmental organizations either national and overseas, with mutual benefit basis in sustainable frame for natural resources conservation in supporting Food Security. The other Unila goals related to the Food Security is the community welfare, in which Unila become the agent of changes and maintain the certainty and justice for the community benefits.

My very sincere appreciation to invited speakers and participants for their great contributions, to all advisory boards SEAMEO-SEARCA and Indonesian Searca Fellows Association (ISFA), reviewers, colleagues and staffs for putting remarkable efforts and their contribution to the organization of this seminar. Finally, I just hope that this seminar is able to inspire and deliver benefits to all participants, in which together we are able contribute to development of Food Security in our countries as well as to global.

We look forward to working with you and getting to know you in years ahead. Thank You.

Your sincerely,

Prof. Dr. Hasriadi Mat Akin

Preface
SEARCA DIRECTOR

MESSAGE

The Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) is pleased to support the Indonesian SEARCA Fellows Association (ISFA) in organizing this *International Seminar on Improving Food Security: The Challenges for Enhancing Resilience to Climate Change.*

SEARCA's support to this event and many similar others is a testament of our commitment to promote food and nutrition security via the route of Inclusive and Sustainable Agricultural and Rural Development (ISARD). Food and nutrition security continues to be a major problem in the region and in the rest of the world in varying degrees and complexities. This is further exacerbated by the impacts of climate change on agriculture which not only serves as the backbone of the economy but is also key to feeding a growing population that continues to struggle with poverty and hunger.

Addressing multi-faceted concerns such as food security and climate change requires collaborative efforts among various stakeholders across the region. That is why SEARCA has developed umbrella programs on food and nutrition security, and climate change adaptation and mitigation which identifies areas for cooperation in research, capacity building, and knowledge management in these two related concerns.

In all these, we are glad to have the cooperation of SEARCA's graduate alumni spread across the region. They have organized themselves into the Regional SEARCA Fellows Association, with at least 8 country chapters including ISFA. The country associations have conducted various knowledge sharing activities such as this International Seminar and plans are also underway for collaborative research projects in the regional alumni organization. By working in synergy, we have seen how the modest contributions of our graduate alumni can make a big difference to agricultural and rural development in the region – truly making them SEARCA's ambassadors in Southeast Asia and beyond.

I congratulate ISFA headed by Dr. Sugeng Prayitno Harianto for organizing this International Seminar which serves as a platform for knowledge sharing on various researches and development activities that contribute to food and nutrition security amidst the detrimental effects of climate change.

Finally, I also thank all our keynote speakers and delegates for their participation in this event and hope to see all of you again in future knowledge sharing events important to the development of the region.

Gil C. Saguiguit, Jr.
Director

KEYNOTES SPEECH

Dr. Siti Nurbaya Bakar
(Minister of Environment and Forestry, Republic Indonesia)

KEYNOTES SPEAKERS

Dr. Ageng S. Herianto, FAO Representative

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Prof. Dr. Neti Yuliana, the University of Lampung

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Dr. Irdika Mansur, Director of SEAMEO-BIOTROP

Prof. Dr. Buhri Arifin, Prince of Songkla University - Thailand

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EFFECT OF COMBINATIONS OF UREA, ZA, AND TSP ON THE GROWTH RATE AND EXTRACELLULAR POLYSACCHARIDE CONTENT OF *Porphyridium* sp.

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ABSTRACT

The purpose of this study was to determine the provision of combinations of urea, ZA, and TSP to the growth rate and extracellular polysaccharide content of *Porphyridium* sp. The study was conducted using a completely randomized design (CRD) with combination treatment of fertilizer: A (25 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP); B (50 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP); and C (75 mg / l of urea: 30 mg / l ZA: 10 mg / l TSP). Control treatment in the study carried out by using fertilizers conwy and apart from the design above. The parameters observed were the density of population, growth rate, and the content of extracellular polysaccharides.

The data were analyzed using ANOVA at α = 5%. The results of data analysis showed that the combination treatment of fertilizer significantly affected the rate of growth, population density, and the content of extracellular polysaccharide *Porphyridium* sp. Growth rate, population density, and the content of extracellular polysaccharide *Porphyridium* sp., sequentially obtained from a fertilizer with a combination treatment of urea concentration 75 mg/l, 50 mg/l, and 25 mg / l.

Keywords: *Porphyridium* sp., growth rate, and poplation density

INTRODUCTION

Porphyridium is a microalgae that has a native habitat in seawater (Vonshak, 1988). *Porphyridium* produces secondary metabolites are excreted in the form of extracellular polysaccharides through the golgi apparatus into the cell culture medium (Kusumawarni 1998). Extracellular polysaccharide produced by microalgae on a stationary phase serves as a protection of cells from unfavorable environmental conditions (Lee, 2008).Growth and

development of microalgae *Porphyridium* influenced by several factors such as temperature, light, salinity, pH and nutrient content in the culture medium (Vonshak, 1988). Vey (1995) explains that the nutrient is one of the growth determinefactors of microalgae in the culture. According to Brown (1997) conwymedia suitable for the growth and development of microalgae in its culture because conwy media containcomplete elements of macro and micro nutrients. In *Nannochloropsis* culture, conwymedia addition of 1 ml/l in the culture produce algal density of 11.08 x 106sel/ml with a cell diameter of 3.19 µm. These results are higher than that which is obtained from microalgae culture containing fertilizers Trace Nutrient Fertilizer (TNF) derived from the decomposition of plant and animal residues. Dose of TNF is 1; 5 and 10 ml/l and each produces the cell density on each peak phase in sequent at 8.33 x 10^6; 10.3 x 10^6; and 5.33 x 10^6sel/ml with cell diameters respectively of 2.18; 3.4 and 3.16 µm (Dayanto *et al*., 2013). The problem of the use of conwy media as a fertilizer or source of nutrients in the cultivation of microalgae is a high price so that the cost burden for farmers to provide fertilizer is very high. Therefore, it needs a fertilizer alternative that costs more affordable, but in accordance with the needs of growing microalgae.

Research on the increase in the growth rate of *Porphyridium* sp. using of agricultural fertilizers has been carried out. Agricultural fertilizer that is used for culturing *Porphyridium* sp.include urea, ZA, and TSP. Nitrogen contained in the fertilizer of urea and ZA also phosphate contained in the TSP fertilizer play a role in increasing the growth rate of microalgae. The addition of nitrogen and ammonium in the culture medium can also increase extracellular polysaccharide content in cultured *Porphyridiumcruentum* (Styaningsih *et al*., 2013). The results of Fogg (1987) indicates that the element of N in the form of nitrate and P in the form of phosphorus are the two main elements that must be present in the culture medium of microalgae. Nitrogen and phosphate nutrients required for the biosynthesis of microalgae protein (Sari *et al*., 2012).

Dose of ZA best in enhancing the growth of *Porphyridium*is 30 mg/l and TSP 10 mg/l. While the dose of urea is most excellent for growing microalgae *Porphyridium* is 50 mg/l (Afriza, 2015). Furthermore Vonshak (1988) states that microalgae, *Porphyridium*, can use KNO3 and ammonium as nitrogen source in its growth.

The purpose of this study was to investigate the combination of concentration of urea, ZA, and TSP which optimal for growth and extracellular polysaccharide content of *Porphyridium* sp.

MATERIALS AND METHODS

Research was conducted in November 2015 until January 2016 at the Laboratory of Aquatic and Botany, Faculty of Mathematics and Basic Sciences, University of Lampung. The tools used are the culture bottles, aerator, paper labels, funnel, plankton-net, aluminum foil, ultraviolet water sterilizer, digital scales, refractometer, pipette, microscope, haemocytometer, measuring cups, cover glass, dark bottle, filter paper , oven, and desiccator.

Materials used are inoculum *Porphyridium* sp. obtained from a stock purely at the Balai Besar pengembangan Budidaya Laut (BBPBL) Lampung which is located in the village of Hanura, TelukPandan, Pesawaran District, Lampung Province; distilled water; alcohol 70%; chlorine; paper towel; sea water; fresh water;media agricultural fertilizer (urea, ZA, TSP), soap; and technical ethanol 96%.

The experiment was conducted using a completely randomized design with 3 treatments. Treatment A (25 mg/l of urea: 30 mg/l ZA: 10 mg/l TSP), treatment B (50 mg/l of urea: 30 mg/l l ZA: 10 mg/l l TSP), and treatment C (75 mg/l of urea: 30 mg/l ZA: 10 mg/l TSP), and each treatment was repeated six times. As a control in this study was the culture of microalgae using the conwy media as a source of nutrition done separately, so it was not included in the analysis of variance and a further test.

Data density of population and the growth rate was analyzed using ANOVA (Analysis of Variance) at α = 5%, while the extracellular polysaccharide content data descriptively explained.

Culture of *Porphyridium* sp.

Culture of *Porphyridium* sp. begins with sterilizing equipment and materials, providing of inoculum and the supply of fertilizers for the treatment. *Porphyridium* inoculum used as much as 1 liter with initial culture density of 150 x 10^6 cells/ml. Inoculum put into culture bottles containing seawater that has been sterilized and fertilized in accordance with the treatment. The calculation of the density of cells under a microscope and using haemositometer done every day for 8 days.

Growth rate

Daily growth rate of microalgae is calculated using the formula:

$$g = \frac{\text{Ln } W_t - \text{Ln } W_0}{t}$$ (Kurniastuty and Julinasari, 1995)

g = daily growth rate (cells / mL / day)

t = time (days) or time of W0 to Wt (cells / mL)

W_0 = initial density (cells / mL)

W_t = final density (cells / mL)

Measurement of Extracellular Polysaccharides Content

Measurement of extracellular polysaccharide content of *Porphyridium* sp. done every day for 8 days. A total of 10 mL samples were centrifuged and the supernatant is then taken. Technical ethanol 96% is then added to the supernatant at a ratio of 1: 1. The mixture was then stored in a freezer for 24 hours. Furthermore, the separation of the polysaccharide from the solution by filtration using filter paper Whitmann. The filter paper is then dried in an oven at a temperature of 45 ° C for 6 hours, then weighed (Styaningsih *et al.*, 2013).

RESULTS AND DISCUSSION

The results of Anova at α = 5% indicates that the difference in the concentration of urea from all treatment combinations in this study (A, B, and C) affects the population density of *Porphyridium* sp. significantly starting on the day 4 (Figure 1).

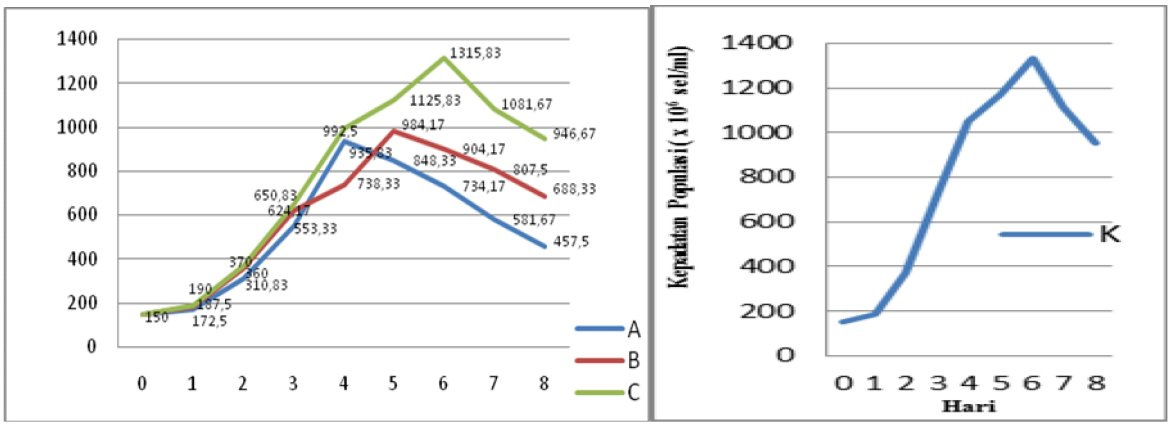

Figure 1. Population density *of Porphyridium*sp. from the age of culture 0 s/d 8 days

Treatment A = 25 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment B = 50 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment C = 75 mg l of urea, 30 mg/l ZA, and 10 mg/l TSP

Controls = fertilizerof conwy 1 ml/l were not included in the analysis of variance and a further test

The fastest exponential phase of the culture *Porphyridium* sp. obtained from the treatment with a urea concentration of 25 mg/l and the slowest obtained from C treatment with urea concentration of 75 mg / l (Figure 1).The data in Figure 1 shows that the lower the

nitrogen content in the culture medium of *Porphyridium* sp. causing faster achievement of the exponential phase, but the period of exponential becoming increasingly shorter.The difference in concentration of urea in the culture medium suspected to affect the speed of achievement of the exponential phase and the period time of exponential phase. This presumption is based on the opinion of Herman et al., (2011) which states that the higher the concentration of urea used in microalgae culture medium causes exponential phase lasts longer.The results of this study are also consistent with the results of Ariza (2015) research which showed that in culture of Porphyridium with urea concentration of 10 mg/l, ZA 30 mg/l, and TSP 10 mg/l, the phase of exponential lasts for 4 days while the culture of microalgae with a urea concentration of 50 mg l, ZA 30 mg/l , and TSP 10 mg/l, the exponential phase lasted for eight days. It is suspected that the high nitrogen content in the culture medium causing prolonged cell division resulting in the highest density cells. This presumptionis based onthe results of this studywith the highest concentration of urea is 75 mg/l reached the highest maximum density with exponential phase longest.

The results of the observations for 8 days, indicating that the growth *Porphyridium* sp. obtained from treatment A with a urea concentration of 25 mg/l gave in the lowest number of maximum cell density, while the number of the highest maximum cell density obtained from C treatment with urea concentration 75 mg/l. Subekti *et al*. (2013) found that the higher the concentration of urea used in the culture medium of microalgae led to the rapid growth of the population so that the population number will increase. In *Nannochloropsis* sp. cultures treated with urea fertilizer ratio of 10 g/l, ZA 20 g/l, and TSP 10 g/l on average produce cell number of 1169.66 x 10^4 cells/ml,whereas cultures treated with the ratio of urea 50 g/l, ZA 20 g/, and TSP 10 g/l to produce a cell number 1371.64 x 10^4 cells / ml.

Table 1. The mean of the *Porphyridium* sp. growth rates as a result of a fertilizer combination treatment

Treatment	**The mean of** *Porphyridium* sp. growth rates**(cells/ml/harday) ±SD**
A	0,123 ± 0,00763 c
B	0,172 ± 0,00918 b
C	0,204 ± 0,00823 a

The number followed by the same letter are not significantly different at LSD, α = 5%

Note: An growth rate mean of the control is 0.205 cells/ml / day

Treatment A = 25 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment B = 50 mg/l of urea, 30 mg/l ZA, and 10 mg/l TSP

Treatment C = 75 mg l of urea, 30 mg/l ZA, and 10 mg/l TSP

According to Herman *et al.* (2011) the growth rate of microalgae is influenced by the media fertilizers used in the culture. While Chrismadha *et al*. (2006) and Widianingsih *et al*. (2008) explains that the nutritional composition in fertilizers that supports the growth of microalgae is the composition of the of fertilizers with a ratio of N: P low. The results of Chrismadha *et al.* (2006) in line with the results of this study, where the rate of growth of phytoplankton or microalgae in culture is limited by the concentration of nitrogen. Nitrogen plays an important role as a constituent amino acids, protein and chlorophyll (Sirappa, 2003).

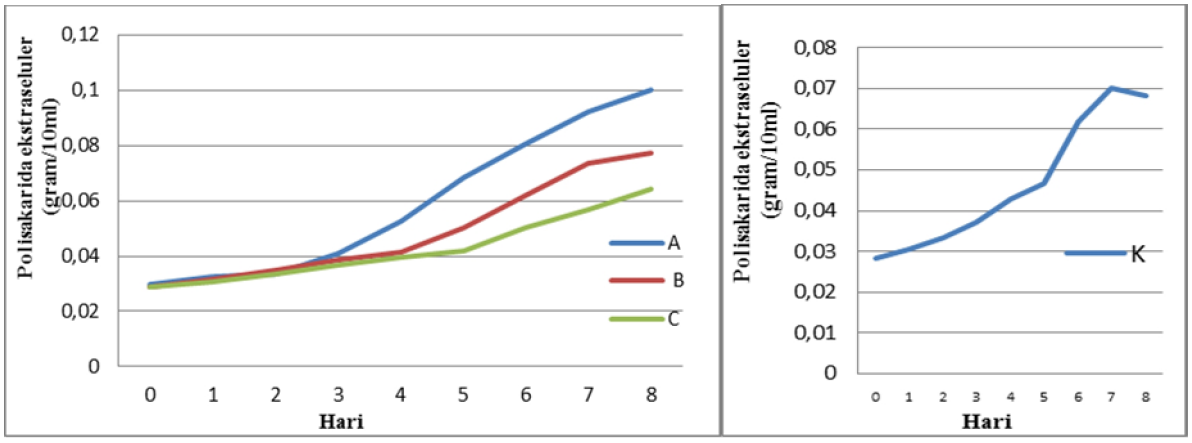

Figure 2. Content of extracellular polysaccharide of *Porphyridium* sp.

In this study, the highest content of extracellular polysaccharides derived precisely from treatment A and the lowest content of extracellular polysaccharide obtained from the C treatment (Figure 2).

According to Fadillah *et al.* (2014) extracellular polysaccharide production by microalgae takes place during the stationary phase.In the stationary phase microalgae *Porphyridium* sp. adapt to unfavorable conditions, ie when the amount of nutrients in the culture medium decreased, by generating extracellular polysaccharide compound (Styaningsih *et al,* 2013). In this study, the stationary phase of *Porphyridium* sp. growth achieved the most rapid obtained from treatment A, but stationary period of treatment A is the longest. In contrast to the culture *Porphyridium* sp. of treatment C that has a shortest stationary period, the stationary phase is achieved at the latest. Thus the period of the formation and excretion of polysaccharides of treatment A is longer and the extracellular polysaccharide produced becomes more.

Different concentrations of urea in combination of several fertilizers significantly affected the rate of growth *Porphyridium* sp. The concentration of urea best to produce high cell is 75 mg/l, whereas the concentration of urea best to produce extracellular polysaccharides is 25 mg/l.

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LIQUID BIO-AMELIORANT AND REDUCTION OF INORGANIC FERTILIZER TO IMPROVE SOIL QUALITY AND MAIZE YIELD

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ABSTRACT

Improving soil quality and plant productivity is still challenging in sustainable agriculture. The purpose of this research was to obtain sustainable crop management in effort to improve soil quality and increase maize yield through the evaliuation of application of liquid bio-ameliorant, reduction of chemical fertilizer, and different planting spaces. Factorial experimental design with three treatment factors and three replications applied and Duncan multiple range test was used to analyze the effects of treatments on all parameters evaluated. The treatments consist of two levels of planting spaces, three levels of liquid bio-ameliorant (LBA) and three levels of nitrogen fertilizer. The results showed that there were improved soil quality parameters such as microbial density and soil chemical properties, which indicated an increase of approximately 28% after the combination treatments of liquid bio-ameliorant and reducing nitrogen fertilizer application. In the same line, a significant improvement in plant growth was shown by the increases in plant height, number of leaves, biomass weight, and content of plant nutrient parameters. The highest maize yield of 9.00 ton/ha was found in the application of 300 ml/l liquid bio-ameliorant and 240 kg/ha urea. It could be infered based on the results that application of liquid bio-ameliorant had a potential to reduce the need for inorganic fertilizers; therefore can be used to sustain crop production and food security.

Keywords: Liquid bio-ameliorant, urea, planting space, soil quality, yields, sub-optimal soil

INTRODUCTION

Maize is an important plant economically and become the second staple food in Indonesia. This figured could be shown from the total of cultivation area around 3,837,000 ha with total production 19,008,426 ton and production demand every year is still increase (National Central Bureau of Statistic/BPS, 2014). In fact low productivity is the main problem of maize production which is the most challenge to fulfill national maize demand. Dry land area with low soil quality especially the limitation in physical, chemical, and biological characteristics were most dominated by area of maize cultivation. Nevertheless, this soil is very potential for the development of maize production because the area of this soil was covered around 12,749,000 ha (Askari, 2010). Efforts to increase maize production can be achieved by adapting three systems as planting management, increasing fertilization and improved varieties. These systems are more specific related to management of the physico-chemical and biological environment and the implementation of appropriate cultivation technology. Maize cultivation was highly dependent to chemical fertilizer, pesticide, and in most cases with the absence of organic material. These practices has been recognized to decrease soil quality and to cause high production cost. The adoption of better management practices (BMPs) can improve soil organic carbon (SOC) content, enhance soil quality, restore degraded ecosystems, increase biomass production, improve crop yield, and encourage investment in soil resources for soil restoration (Lal *et al*., 1998).

Agriculture by products such as organic materials are often found in agricultural production area, which in many cases they become wastes. The other organic material source comes from liquid (sludged) waste of biogas product. Both sources have the potential to produce materials for liquid bio-ameliorant (LBA), through anaerobic fermentation process (Setiawan, 2010). Combining the application of organic bio-ameliorant with chemical fertilizers could be an alternate practice in order to optimize maize yield and maintain soil productivity. Benítez-Noyola (2013) demonstrated that maize plants fertilized with 90 and 180 kg N ha^{-1} and inoculated with *Paenibacillus polymyxa* produced 20 to 28% more nitrogen content and higher grain yield than those only fertilized with inorganic fertilizers. Naveed *et al*.,(2008) reported that it was possible to maintain maize yields by replacing 87 kg urea ha^{-1}, which was (50%) of the complete N fertilizer dose (175 kg ha^{-1}) with 300 Kg ha^{-1} of organic compost from fruit and vegetable wastes, enriched with 147 g N fertilizer kg^{-1} compost. This study aimed to obtain better maize cultivation technology which results in an

improved soil quality and increased yield through the combination application of LBA, reduction of nitrogen chemical ferilizers and crop management. Better knowledge and considerable information on maize cultivation management practices under integrated models are needed to generate effective strategies to achieve an improved soil quality and sustainable agriculture.

MATERIALS AND METHODS

The field experiment was carried out in Teaching Farm, Faculty of Agriculture, Hasanuddin University. The experiment was set up in a factorial experimental design replicated three times in 2x2 m field plots. Treatments were arranged in three factors, i.e., (1) planting space with two type spaces (P_1=75 x 25 cm and P_2=50 x 20 cm); (2) liquid bio-ameliorant (LBA) with three concentrations (B_0=0 ml/l; B_1=100 ml/l; and B_2=300 ml/l); and (3) urea fertilizer with three levels (N_0=0 kg/ha; N_1=300 kg/ha; and N_2=240 kg/ha). Preparation of the LBA was done by mixing all organic materials containing sludge of biogas, lake organic material sediment, and plant by-products. This mixing organic material was collected and extracted into biodigester in 100 L capacity. Development of biological activity of this materials was performed by the use of molasses, coconut water, and local microorganisms isolated from compost as bioactivators. This process is carried out in three (3) biodigester with different composition formulation of in each biodigester. The LBA was ready for use after approximately four week- incubation. The preparation of LBA, soil and plant analyses were conducted at the Laboratory of Soil Chemistry and Fertility, Department of Soil Science, Faculty of Agriculture, Hasanuddin University, Makassar.

The LBA was applied at two weeks after planting and repeated every two weeks during vegetative growth period of maize. Nitrogen fertilizer treatments were applied in split three times: 40% in 7 days after planting (DAP), 30% in 30 DAP, and 30% 50 DAP. Furthermore, all plots were supplemented with 150 kg/ha super phosphate (SP36) and 100 kg/ha potassium chloride (KCl).

Soil samples for soil chemical property analyses were collected as composite samples from mixing soil of both planting space types. So, the soil samples were distinguished based on nitrogen fertilizer and LBA treatments. Basic soil analyses were conducted as followed. Soil pH was measured in a 1:2.5, soil:solution ratio (in both water and 1 M KCl) using a glass electrode. Exchangeable cations were extracted with 1 M ammonium acetate (1:50 soil

extractant ratio for 2 h) and the filtered extract was analysed for Ca, Mg, K and Na by atomic absorption spectrophotometry. Total Nitrogen by Kjeldahl digestion with colorimetric determination of liberated NH_4^+ (Foster, 1995), and organic C bythe Walkley and Black dichromate oxidation procedure (Blakemore *et al*., 1972). Plant samples were digested in nitric and perchloric acids and the phosphorus content of digests was measured by the molybdenum blue method (John, 1970). All data were subjected to analysis of variance (ANOVA) and the effects of different treatments were evaluated using Duncan's multiple range test. All statistical analyses were performed using MStat-C computer software.

RESULTS AND DISCUSSION

1. Change in Soil Quality

Maize cultivation is highly depended upon the soil fertility and other environmental conditions. In the soil with low nutrient content, especially nitrogen, maize yield could be drastically reduced. Long termed application of inorganic fertilizer tends to decrease soil quality which in turn directly affects the environment as well as plant productivity. In such cases, a combined method to maintain sustainability of crop production could be achieved by modifying the environment, for instance by application of organic materials such as LBA. Results of this experiment showed, that application of LBA had significant effects in the soil chemical properties, as shown in Table 1. It was shown that application of LBA was able to reduce utilization of chemical fertilizers.

2. Plant Performance

Application of LBA and nitrogen fertilizers significantly affected total plant height, both as single and as combination treatments. However, planting spaces applied resulted in the same plant heights (Table 2).

The highest result in the total plant height was performed in the treatment 100 ml/l LBA with 300 kg/ha urea (B_1N_1). However, those figures of plant height were not significantly different from those obtained by the application of 300 ml/l LBA and 240 kg/ha urea (B_2N_2). Effects of planting space on the plant performance showed that generally the treatment with 50 x 20 cm (P_2) was better than 75 x 25 (P_1) cm planting spaces. In overall treatments, plant height was significantly lower in the single treatment application of either of LBA or urea fertilizer. The difference in plant height indicated that LBA could be applied as

a complement to nitrogen fertilizer. The results also suggested that higher concentration of LBA and reduced nitrogen fertilizer resulted in the same effect with lower concentration of LBA and higher nitrogen fertilizer.

Table 1. Change of soil chemical properties after plant harverst

Treatments	Soil Chemical Properties								
	C	N	P_2O_5	K	Ca	Mg	Na	KTK	KB
	%	%	ppm	cmol/kg	cmol/kg	cmol/kg	cmol/kg	cmol/kg	%
N_0B_0	1.94	0.06	10.99	0.22	6.36	2.67	0.33	18.34	52
N_0B_1	1.75	0.34	36.87	0.25	8.38	4.87	0.41	22.33	62
N_0B_2	2.49	0.36	37.82	0.36	7.90	3.50	0.38	24.33	50
N_1B_0	2.14	0.31	18.48	0.17	6.83	4.63	0.33	28.51	42
N_1B_1	2.59	0.08	34.99	0.25	7.78	3.03	0.42	22.13	52
N_1B_2	2.59	0.28	35.74	0.22	7.48	2.79	0.61	24.13	46
N_2B_0	2.71	0.22	10.00	0.22	6.83	2.85	0.35	22.53	46
N_2B_1	2.24	0.24	34.99	0.24	8.49	0.42	0.52	21.34	45
N_2B_2	2.70	0.21	34.04	0.33	8.08	1.60	0.41	21.14	49

Plant biomass was obtained after harvesting and susequently dried in 70^{o}C for 48 h for dry weight determination. Combination treatments of 100ml/l LBA, 300kg/ha urea, and 75 x 25 cm planting space resulted in the highest dry weight (Table 3). In general, the results showed that that treatments without LBA or without nitrogen fertilizer produced lower biomass weight than those produced in the combination of both treatments. An interesting result also found that higher concentration of LBA (300 ml/l) and lower nitrogen fertilizer (240 kg/ha) could maintain high biomass production.

Table 2. Effects of liquid bio-ameliorant, nitrogen, and planting spaces on total plant height (cm).

Treatments	Average(cm)	Note*
$P_1B_1N_1$	198,07	a
$P_2B_1N_1$	185,60	a
$P_2B_0N_1$	184,64	a
$P_1B_2N_2$	183,57	A
$P_2B_2N_2$	183,55	A
$P_2B_1N_2$	183,00	A
$P_2B_2N_1$	178,86	ab
$P_2B_0N_2$	177,90	B
$P_1B_1N_2$	177,33	B
$P_1B_0N_1$	176,03	B
$P_1B_0N_2$	171,43	B
$P_1B_2N_1$	167,90	B
$P_2B_1N_0$	157,65	bc
$P_2B_0N_0$	153,19	C
$P_2B_2N_0$	150,55	C
$P_1B_2N_0$	143,50	C
$P_1B_1N_0$	137,77	C
$P_1B_0N_0$	134,37	C

*The same letter in the note colum was not significantly different at 1% Duncan's test

Table 3. The Differences plant biomass weight (g) in liquid bio-ameliorant, nitrogen fertilizer, and planting space treatments.

Treatments	Average (g)	Note*
$P_1B_1N_1$	124.00	A
$P_1B_0N_2$	119.27	A
$P_1B_1N_2$	110.83	A
$P_2B_2N_2$	109.43	A
$P_1B_2N_1$	98.77	A
$P_2B_2N_1$	96.97	A
$P_1B_0N_1$	96.37	A
$P_2B_0N_1$	85.60	Ab
$P_1B_2N_0$	82.37	B
$P_1B_2N_2$	80.00	B
$P_2B_1N_2$	75.47	B
$P_2B_1N_1$	67.27	B
$P_2B_1N_0$	61.53	B
$P_2B_2N_0$	57.57	B
$P_2B_0N_0$	56.83	B
$P_1B_1N_0$	54.00	B
$P_1B_0N_0$	53.57	Bc
$P_2B_0N_2$	51.50	C

*The same letter in note column was not significantly different at 1% Duncan's test

3. Plant Nutrient Uptake

Plant nutrient is an important indicator in soil–plant relationship. In the soil with high nutrient availability, it is easy to trace the nutrient plant content. The amount of nutrient uptake (nitrogen and phosphorus) in all treatments were shown in Table 4. Nitrogen and phosphorous uptake by plants were significantly affected by different treatments assigned. The higher contents of plant nutrient uptakes were dominated by combination treatments of LBA and nitrogen fertilizer. Planting space in 75 x 25 cm produced higher nutrient uptake than that in 50 x 20 cm. The results also showed the uptake of nitrogen and phosphorous by plants were relatively higher in LBA treatment without nitrogen fertilizer application.

4. Yields

Yield illustrates the working interactions among various factors which determine all production process including soil, fertilizers, and the plant environment. Total maize yield in this experiment was shown in Table 5. The highest yield was obtained from combination treatments between 300 ml/l LBA and 240 kg/ha nitrogen fertilizer with 50 x 20 cm planting space.

Table 4. Nitrogen and phosphorus uptake by plants in all treatments assigned.

Treatments	Amount of Nutrient Uptake	
	N (%)	P(ppm)
$P_1B_1N_1$	3.76	1.36
$P_2B_2N_2$	3.60	1.01
$P_1B_0N_2$	3.02	1.14
$P_1B_1N_2$	2.47	1.03
$P_1B_2N_2$	2.47	0.76
$P_2B_1N_2$	2.32	0.79
$P_2B_2N_1$	2.18	0.88
$P_1B_0N_1$	1.86	0.88
$P_2B_0N_1$	1.73	0.79
$P_1B_2N_1$	1.66	1.03
$P_2B_0N_2$	1.64	0.48
$P_2B_1N_1$	1.53	0.61
$P_1B_2N_0$	1.36	0.75
$P_2B_2N_0$	1.34	0.49
$P_2B_1N_0$	1.06	0.57
$P_2B_0N_0$	0.77	0.54
$P_1B_1N_0$	0.73	0.48
$P_1B_0N_0$	0.30	0.48

The result also showed that LBA might improve the soil environment by reducing the need for nitrogen fertilizer. This result confirmed the advantage of LBA as a material that could be used to improve maize production, especially in sub–optimal soil.

Table 5. Total maize yields in different combination of treatments.

Treatments	Yields (kg) (in plots 2 x 2 M)	Yields (ton/ha)
$P_1B_1N_1$	5.40	5.32
$P_2B_2N_2$	7.92	9.00
$P_2B_0N_1$	3.24	3.12
$P_1B_0N_0$	2.64	3.00

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The combination treatments of low LBA concentration and high nitrogen fertilizer application resulted in better plant performance; However, the highest maize yields were obtained in the combination treatments of high LBA concentration and low nitrogen fertilizer. These results confirmed the advantage of LBA as a fertilizer complement that reduce the need for inorganic fertilizer.

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SOIL RESOURCE INFORMATION SYSTEM OF CAGAYAN VALLEY

A Guide for a Sustainable Agricultural Production System

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ABSTRACT

A soil resource database of the major agricultural soils of Cagayan Valley was developed and complimented by a printed guidebook "Soils in Cagayan Valley: Guidebook to Sustainable Soil Resource Management". The database contained an inventory of all the established soil series in the four provinces of Cagayan Valley, Region 2– Cagayan, Isabela, Nueva Vizcaya and Quirino. Morphological, physical and chemical properties of the established soil series of the four provinces under study were collated and properly validated in the field using standard procedures of profile description, soil sampling and laboratory testing. After correlation and validation of the primary and secondary data, assessment of crop suitability was conducted, and finally,management recommendations for each soil series were formulated. The locationsof the soil at the barangay level were identified using the Geographic Information System (GIS).

The soil resources database includes the soil physical and chemical characteristics, constraints to crop production, suitability for major crops of the region, and soil management recommendations intended for agricultural practitioners to grasp vital information on the different soils in the region. It will guide technicians and farmers to undertake decisions on what crop to grow in a specific soil series based on crop suitability rating, harnessing the innate characteristics and nutrient contents of the soils, as it provides standardized systems in nutrient management stipulating the quantified fertilizer inputs for specific soil series – an information needed at the field for optimal and sustainable soil resource management.

As designed, searcher can select a specific province, then municipality and then a particular barangay where he wants to identify its dominant soil series. A user friendly system would facilitate the location of the soil in any of the four provinces as well as show the municipalities under specific province. A list of barangayscan be seenas well and the selection for specific barangay can therefore be obtained easily. Once the barangay is selected, the dominant soil series in that area with the corresponding information on their

characteristics, crop suitability, limitations up to soil management recommendation will be seen on the screen.

The guidebook will complement the information system, especially for localities lacking tools to access the system. The information contained in the soil resource information system can therefore serve as decision-support information system towards sustainable agricultural production.

Keywords: *Soil survey and classification, Geographic Information System (GIS), soil series, crop suitability, land evaluation, soil database, Cagayan Valley*

I. RATIONALE/SIGNIFICANCE OF THE STUDY

Agriculture is the principal industry in the Philippines and it remains as the main livelihood of millions of Filipinos. Its advancement and sustainability depends much on the proper conservation of soil resources of the country. However, so much activity remains to be done to improve the agriculture industry and to increase the earning capacity of the farmers. Every year there is an anticipated shortage of the staple food crops in the country because of continuous decline in the quality of our prime agricultural areas. The soils in most of our arable areas have been seriously depleted of nutrients primarily due to the practice of nutrient mining by majority of our poor Filipino farmers. Such practice therefore requires immediate and continuous replenishment of the depleted nutrients in order to revive as well as sustain soil productivity. More and more of the marginal lands located in the rolling and hilly lands have been brought to tillage in order to augment declining productivity of the remaining prime agricultural areas in the country. The opening of marginal areas by mostly marginal farmers, farmers without the capacity to provide adequate inputs as well as lacking sufficient land management skill and field experiencewould inevitably result into occurrence of serious soil erosion and may even endinto irreversible land degradation.With the advent of new technologies, it is believed that the present area devoted to food crops can be made to produce much more than the need of the population of the country today. One way of raising productivity of existing agricultural areas would be the utilizationof land according to its best use.This can be achieved through proper understanding of the production capacity of soils. The nature and properties of soils as they occur in the field including the recognition of their

respective constraints to crop production come under the scope of soil survey and classification activities.

Soil surveying and classification is the science of studying characteristics and distribution of soils in the field. It has been designed to help farmers identify and characterize the soils in the farm. Further, through soil surveying, the behavior of different soils when grown to different crops is determined including the inherent limitation of the soils to different uses. The result of evaluation of the morphological, physical, chemical characteristics and inherent limitations of the soilsto various uses can guide in the establishment of proper use and management of soils. It can likewise help in selecting the most suitable crops to grow relative to the kind of soil. Unfortunately, the use of soil survey and soil classification is seldom realized primarily because of the problem of presentation of the report which is highly technical and sometimes too complicated to ordinary farmers, not to mention the problem of availability, accessibility and the recency of information.

This project is an attempt to translate and simplify the soil resource information generated from the soil survey and classification activity and to make it accessible to the farmers, agricultural technicians and researchers. Access to the said information can be made possible through the use of internet technology. The development of information systemcovers a comprehensive database related to description, characteristics, crop suitability, limitations and behavior of the major agricultural soils in Cagayan Valley. It can be accessed by farmers, technicians, researchers and other end-users and can be used for proper soil identification and characterization. Through a users friendly system, they can acquire the information about the soils in their locality, they can easily exchange information related to farm problem identification, and can cooperate in finding solution to the problem as well, especially in matters related to the selection of appropriate crop and recommended agricultural production system. With the soil seriesidentified up to barangay level, the information system can likewise serve as vehicles of transfer for site specific technologies. Further, allocation and optimization of land use especially in the selection of crop/cropping pattern/production system can be done more systematically.

II. OBJECTIVES

To develop a soil information system that contains a comprehensive soil database related to description, characteristics, crop suitability, limitations, recommended management

strategies, and behavior of major agricultural soils in Cagayan Valley. Specifically, it aimed to:

a. To validate and update existing data on the morphological, physical and chemical characteristics of the major soil series in Cagayan Valley;
b. To collate available information related to the agro-ecological distribution and behavior of major soil series when used for crop production
c. To develop tailor-made printed and automated guide to soil identification and characterization up to barangay level; and
d. To offer guides for fertilizer requirements, fertilizer material equivalents and lime requirement for the major soils of the valley when grown to the 11 major crops of the region.

III. METHODOLOGY

The information system contains inventory of all the soil resources of the four provinces – Cagayan, Isabela, Nueva Vizcaya and Quirino. The project essentially involved six general activities: (1) collection, collation and interpretation of available secondary data; (2) correlation and validation of the soil morphological description; (3) determination of physical and chemical properties of the soils; (4) taxonomic classification of individual soils at series level; (5) webpage design, development of soil resource information system and formulation of the guidebook; and, (6) pre-evaluation, training and dissemination of information about the webpage and the guidebook.

Assessment of data requirements and study site

The project relied on available records about the characteristics, behavior and distribution of major soils series in Cagayan Valley. It primarily depended on published Provincial Soil Survey Report prepared by the Bureau of Soils and Water Management (BSWM), including other relevant data and information. Established soil series were categorized according to their importance to crop production and geographic distribution.

Systematization and interpretation of the soil survey reports

Following the analysis of available information related to the characteristics and distribution of major soil series, the researchers located on a controlled maps the reported places where the individual soil series in the region were mapped. Further, a genetic key that

relates unique individual features and landscape distribution was prepared to simplify the identification of major soil series in the field.

Digging of pits, soil characterization and collection of samples

Next to collection and analysis of secondary data was the field validation of target soil series. Geographic positioning system (GPS) was used to obtain the geographic reference sites of the major soil series of the region. Soil pits which measured one-meter wide and one-meter long were dug at a depth of about 1.5 meters to fully expose the soil profile which were described according to the guidelines of the USDA. During field validation, the morphological characteristics of the known soil series were examined, described and pictures taken from a newly opened pit. In addition, soil samples per identified soil horizon/layer were taken for laboratory analysis.

Sample collection, preparation and laboratory analysis

Soil samples were collected from each horizon of the soil profile, air-dried crushed, thoroughly mixed and passed through a 2.0 mm sieve for physical and chemical analysis. The current and potential suitability ratings of the major soil series were determined by matching the soil qualities with the crop requirements. The suitability assessment adopted the three classes within the order S for suitable and two classes within the order U for unsuitable as provided in the FAO framework.

Database Encoding, Webpage Design and Development

Further processing of both secondary and primary data collected during field validation was conducted preparatory to the development of the soil information system (webpage) and the guidebook. The step required the continuation of correlation between the secondary data and primary data. Secondary data on morphological description and soil properties were matched with the primary data to confirm the validity of the information. Finally, all data and information of soil series were organized into a soil information system that include description of the major soil series, soil quality, suitability ratings and limitations, recommended management practices and fertilizer guides.

Pre-test of the soil information system of the region

In the validation of the soil resource information system, the researchers invited the municipal agriculturists, technicians, extension workers and other interested private groups

including farmers from the area to give comments, suggestions and recommendations regarding the content, color and texture, font style and format of the developed webpage and guidebook. The system and the guidebook were presented to the participants who were then asked to fill-up a set of questions provided in a pre-tested questionnaire.

IV. HIGHLIGHT OF ACCOMPLISHMENT

A. THE INFORMATION SYSTEM (IS)

The project output, "**Major Soils of Cagayan Valley: A Land Resource Information System**" is a software by which farmers, extension workers, agricultural technicians, researchers, and other interested parties can use as a guide to identify a soil series, the morphological, physical and chemical properties, featurestranslated as land qualities, their crop suitability, inherent limitations, recommended management strategies, fertilizer requirement and equivalents. Further, soil series at barangay levels can be identified complemented by soil profile description and picture which can serve as a guide for proper soil identification.

Soils of the Region

There were thirty major soil series identified and field validated in Cagayan Valley. These included the fifteen lowland soils occupying the coastal plains, flood plains and undulating areas. All the soils were developed from alluvial deposits and they comprise the most productive agricultural areas of the valley. These include the *Bago, Bantog, Barcelona, Bigaa, Brooke's, Buguey, Isabela, Maligaya, Quingua, San Fernando, San Manuel, Sta. Rita, Toran, Umingan* and *Zaragoza series*.

Fifteen soil series were located in the undulating to rolling and hilly areas of the region. These soils of the uplands, hills and mountains are derived through the weathering of the various igneous rocks, shale calcareous sandstone coralline limestone. They are generally medium to fine texture soils and exhibit a wide range of colors predominantly by brown, reddish brown, red and black. These are *Alaminos, Annam, Bantay, Bolinao, Carig, Cauayan, Faraon, Guimbalaon, Ilagan, Luisiana, Rugao, San Juan, Sevilla, Sibul*and *Sta. Filomena series*.

Land Qualities of the 30 Soil Series

Land quality is a complex attribute of land which affects its suitability for specific uses. It is formulated to avoid referring to a large number of individual characteristics in land evaluation e. g. mean annual rainfall, soil water-holding capacity, and slope. Examples of land quality are topography, susceptibility to flooding, drainage property, soil reaction, availability of nutrients, and susceptibility of soils to erosion. Land quality can be related to the performance of crop yields as influenced by soil properties such as relief, drainage, effective rooting depth, texture, soil pH, organic matter content and CEC. On the other hand, crop requirements such as the soil depth, texture, slope, drainage and pH can be interrelated to the land quality such that the performance of the crops can be analyzed.

Clicking this icon will show the land qualities and characteristics of the identified 30 major soil series of the valley that include the morphological features, physical and chemical properties. Knowing the physical, chemical and morphological features of the soil leads to its proper use and management.

Crop Suitability and Limitations

Based from the physical and chemical properties of the different soil series in Cagayan Valley, the ratings for the limitations and suitability for the 11 major crops of the valley was determined based on slope (t), drainage and flooding (w), soil depth, texture (s) pH and percentage organic matter (f). The FAO land evaluation system was used in determining the crop suitability which was interpreted based on limitations of the different soil series to crop production. The system adopted the use of three classes within Order S and two classes within Order U.

This information guides technicians and farmers as well, to undertake decisions on what to grow on a specific soil series based on the standard crop suitability; harnessing the innate characteristics, and nutrients of the soils.

Suitability Maps and Area Distribution

In response to the food security program of the government, arable lands were delineated and suitability maps were generated based on the result of land evaluation undertaken. Under this category are the suitability maps and distribution of the suitable areas for the eleven major crops of the region that include: lowland and upland rice, corn, banana, mango, pineapple, sugarcane, coconut, tobacco, peanut and cassava.

The maps will guide the user on the specific location of soils considered highly and moderately suitable for a particular crop.

Fertilizer Guides

This section utilized standardized systems in nutrient management stipulating the quantified fertilizer inputs for a specific soil series – information needed at the field for optimal and sustainable soil resource management. With the scenario in the field having farmers implement fixed amount of fertilizer in their fields without informed decisions as to the characteristics and nutrient supplementation needs of their lands, the gap to answer is the provision of information system stating the required input supplementations to cut agricultural cost by utilizing the inherent potentials of the various soil series.

Soil Series Determined at Barangay Levels of the Four Provinces

The location (provincial, municipal, and barangay level) of the soil was identified using the Geographic Information System (GIS). The user can select from the four provinces – Cagayan, Isabela, Vizcaya and Quirino. The various municipalities within a province will be provided with the respective list of the barangays. The user can then select the barangay he/she wishes to identify associated soil series and their characteristics. Once the barangay is selected, the soil series with a representative soil profile will be shown on the screen along with corresponding information on its physic-chemical characteristics, suitable crops, suitability ratings and limitations, and soil management recommendations.

B. THE GUIDEBOOK

The guidebook "Soils in Cagayan Valley: Guidebook to Sustainable Soil Resource Management" provides a holistic approach towards achieving sustainable soil resource management – a decision-support system for agricultural practitioners to discern effectively in terms of managing and harnessing the bounties offered by one of the most indispensable agricultural commodity – the soil.

The book is intended for agricultural practitioners to grasp vital information as to the characteristics, properties, crop suitability, limitations and recommended management strategies of the different soils in region 2. The book guides technicians and farmers as well to undertake decisions on what crop to grow in a specific soil series based on standard crop suitability rating, harnessing the innate characteristics and nutrient contents of the soils, and

finally, it provides standardized systems in nutrient management stipulating the quantified fertilizer inputs for specific soil – an information needed at the field for optimal and sustainable soil resource management.

Information derived in the Soil Information System

The bottom line of knowing the soil series in a specific area and its properties is to determine soil management required to attain higher agricultural productivity and assure sustainability. Information on soil characteristics give us idea on the soil features that limits crop production, consequently determining what are the appropriate management practices needed to conquer such limitations. Moreover, information on soil qualities and soil requirements of crops, and suitability analysis of the major crops commonly grown in the valley was done and indicated in the information system. Accordingly, fertilizer and lime recommendation were derived on specified crops for each of the 30 major soil series. Crop suitability analysis provides information on soil properties that limits the production of specified crop(s). For instance, growing corn in Bantog clay loam would have problems on wetness or flooding indicated by the subscript, ***w***. Further, it shows what crops that would give the highest benefit in terms of productivity and profitability from a given soil series, indicated by S_1 as the most suitable down to S_3 as marginally suitable. The symbol ***U*** implies that the crop is either currently not suitable, (U_1) where the effect of limitation is so severe as to greatly reduce the yield or to require costly inputs, or permanently not suitable (U_2) where the limitations cannot be corrected permanently.

The main component of the project was the development of soil resource information system in printed and automated forms for Cagayan Valley. The developed information system reinforced by the guidebook was designed for use by the municipal agriculturists, technicians, extension workers, farmers, researchers and other interested parties. It includes information about general characteristics of the different soil series found within the region together with their classification. The proper identification of soil series up to barangay level is important for it can improve communicating farm related problems between the technicians and farmers.

Ultimately, the project – soil information system of Cagayan Valley - aims to provide a holistic approach towards achieving sustainable soil resource management – a decision-support system for agricultural practitioners to discern effectively in terms of managing and

harnessing the bounties offered by one of the most indispensable agricultural commodity- the soil.

ACKNOWLEDGMENT

The development of this land resource information system of the major agricultural soils of Cagayan Valley was made possible through the funding support of theSoutheast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) through the Re-entry Program of the Research and Development Unit. The researcher wishes to express his heartfelt gratitude and sincerest appreciation toDr. Gil C. Saguiguit, Dr. Mercedita A. Sombilla, Ms. Ruby Johnson and Ms, NyrhiaRoguel.

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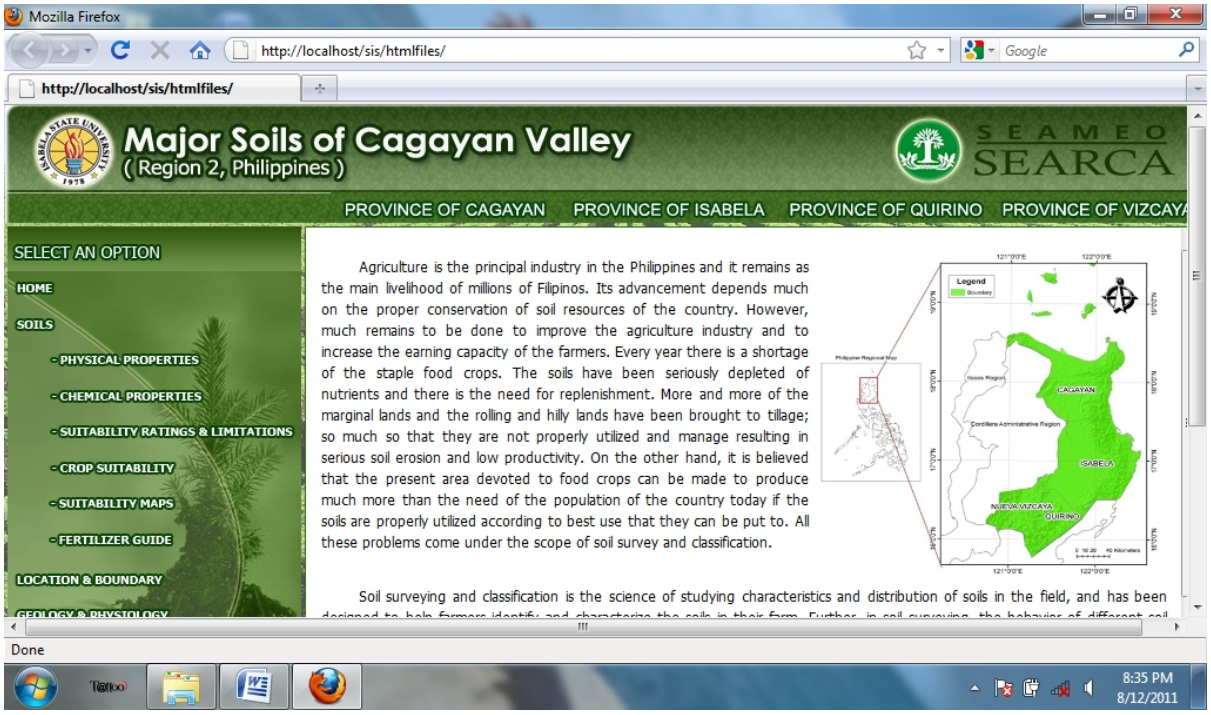
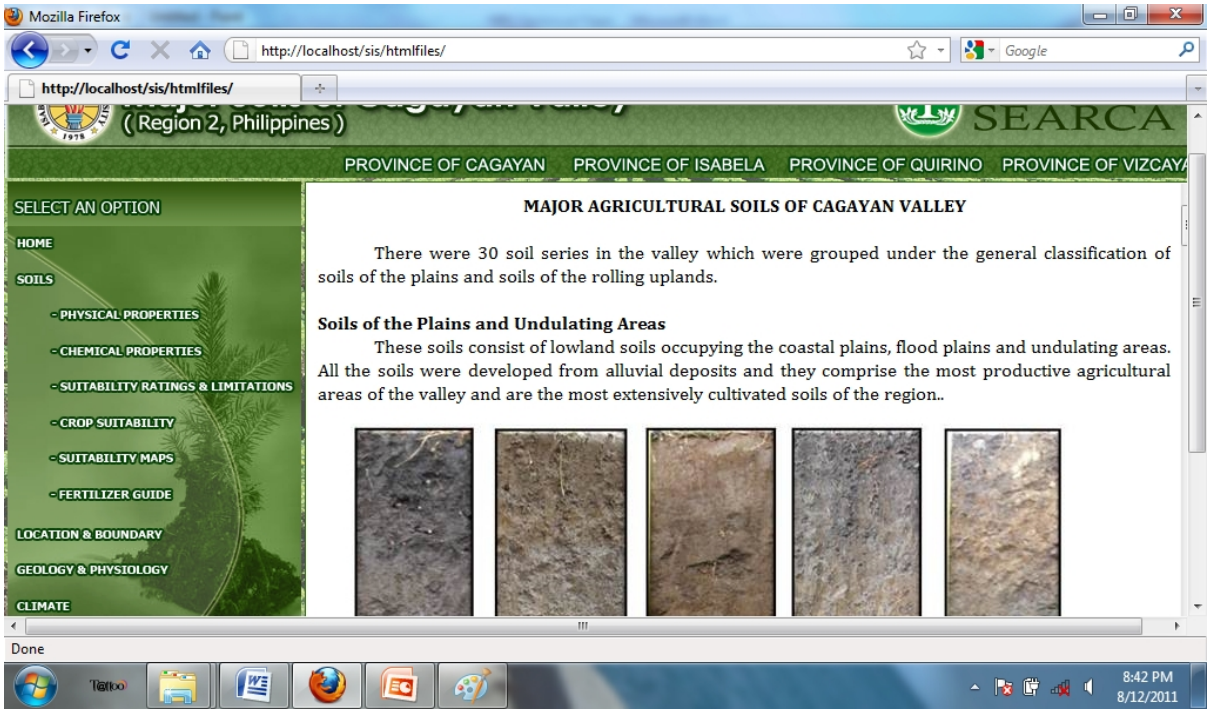
APPENDICES

Overview of the Information System (IS)

HOME – About the IS



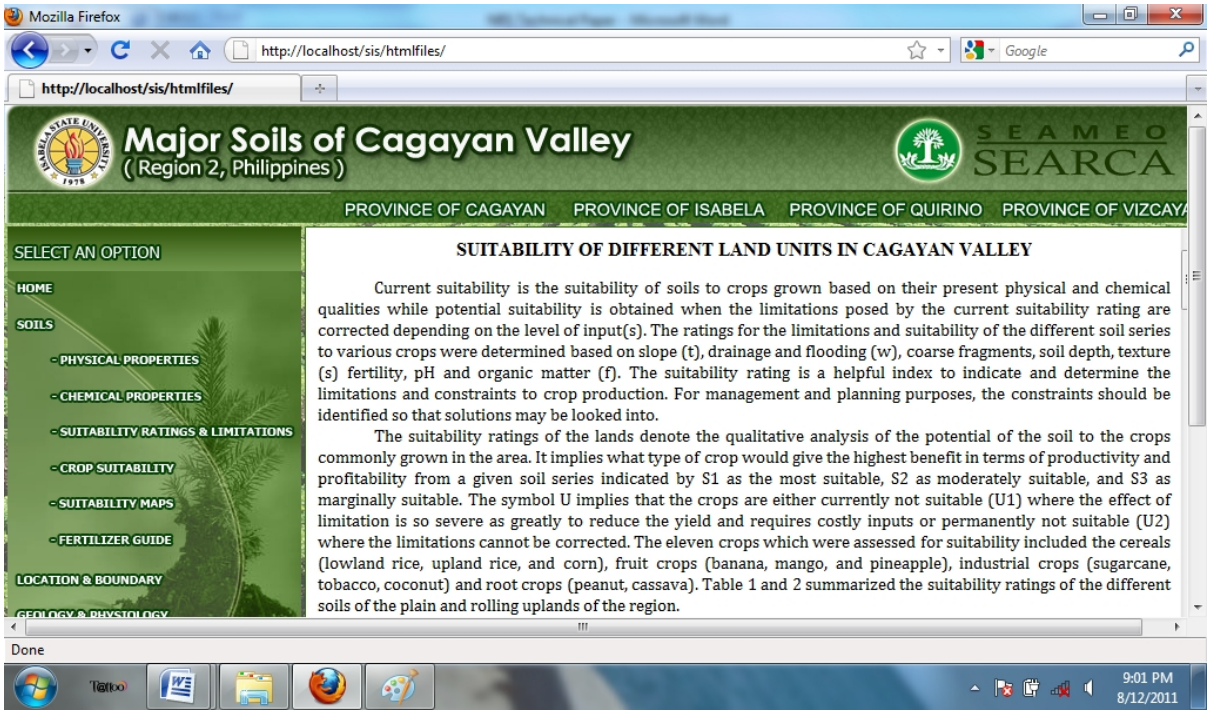
Soils of Cagayan Valley



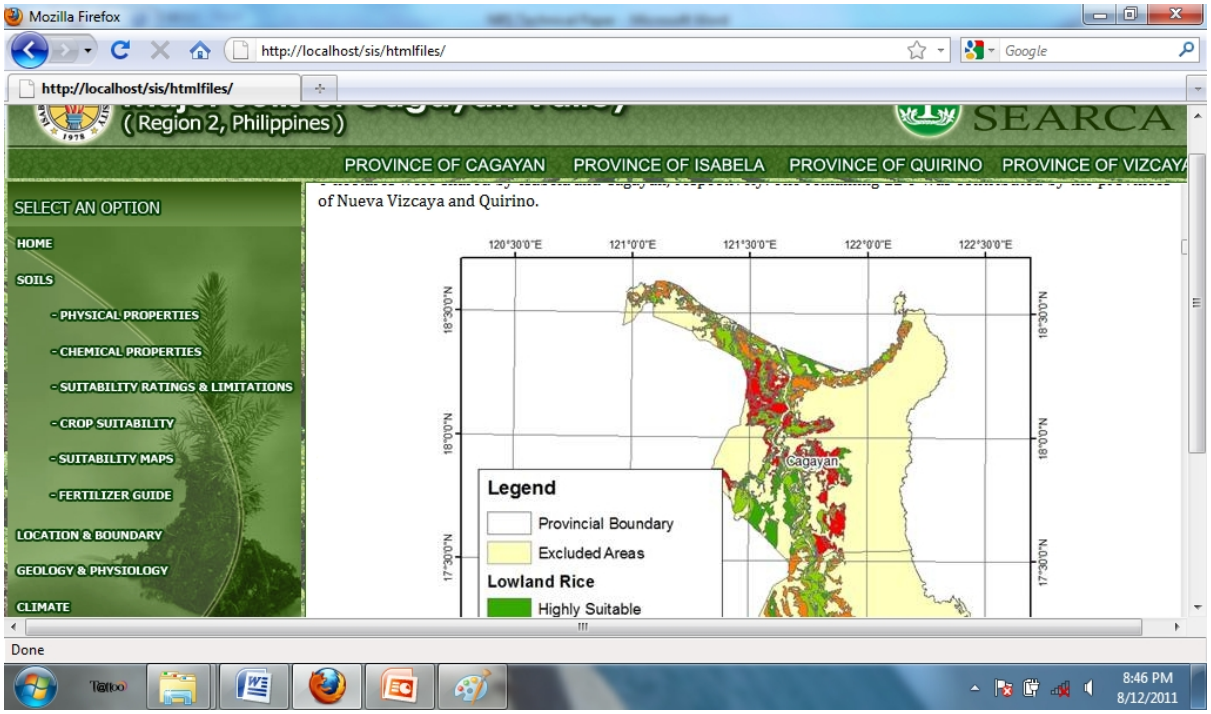
Suitability Ratings and Limitations of the Different Land Units

Soil Series	Lowland Rice	Corn	Upland Rice	Banana	Sugarcane	Mango	Pineapple	Coconut	Tobacc
Bago	S1	S2w	S3w	S3w	S2w	U1sw	S3w	S3w	S3w
Bantog	S1	S2w	S3w	S3w	S2w	S3w	S3w	S3w	S3w
Barcelona	S1	S2w	S2w	S2w	S2w	S2ws	S2w	S2w	S2w
Bigaa	S1	S2w	S3w	S3w	S2w	S3w	S3w	S3w	S3w
Brooke's	S1	S3w	S3w	S3w	S3w	S3w	S3w	S3w	S3w
Buguey	S3s	S3s	S3s	S3s	S3s	S2s	S2s	S1	S1
Isabela	S1	S2w	S3w	S3w	S2w	S3w	S3w	S3w	S3w
Maligaya	S1	S2w	S3w	S3w	S2w	S3w	S3w	S3w	S3w
Quingua	S2w	S1	S1	S1	S1	S1	S1	S1	S1
San	S1	U2w	U2w	U2w	U2w	U2w	U2w	U2w	U2w

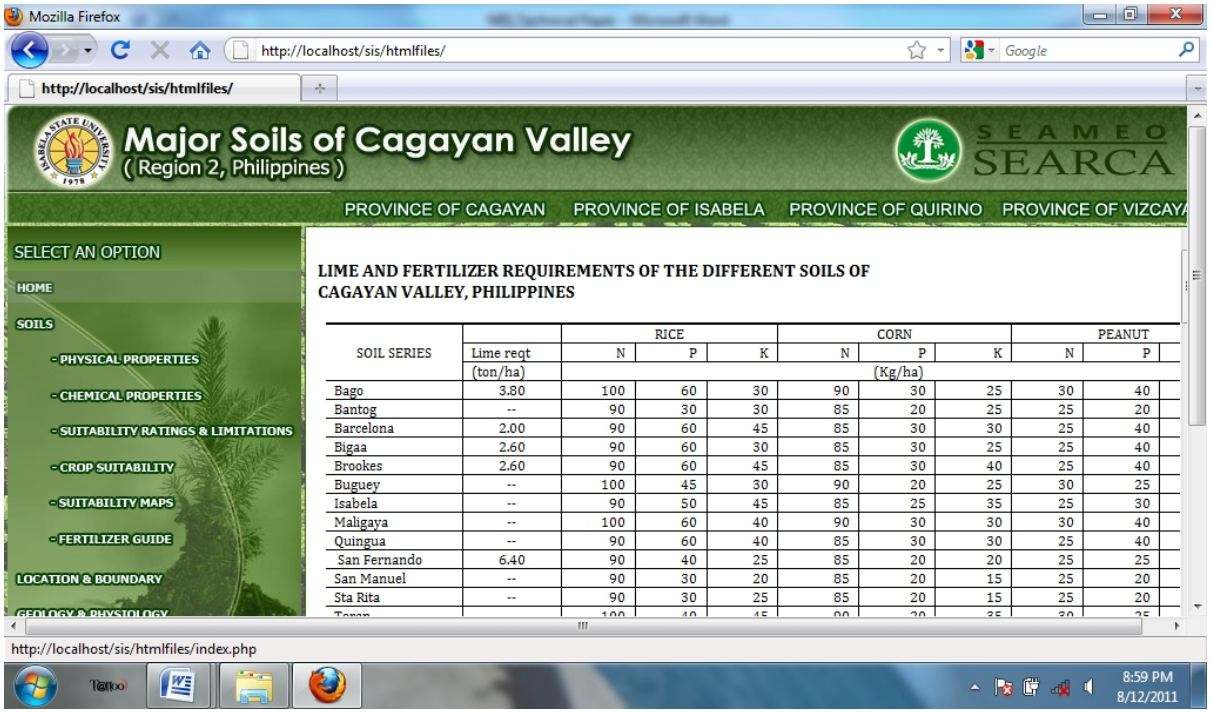
Crop Suitability



Suitability Maps and Area Distribution

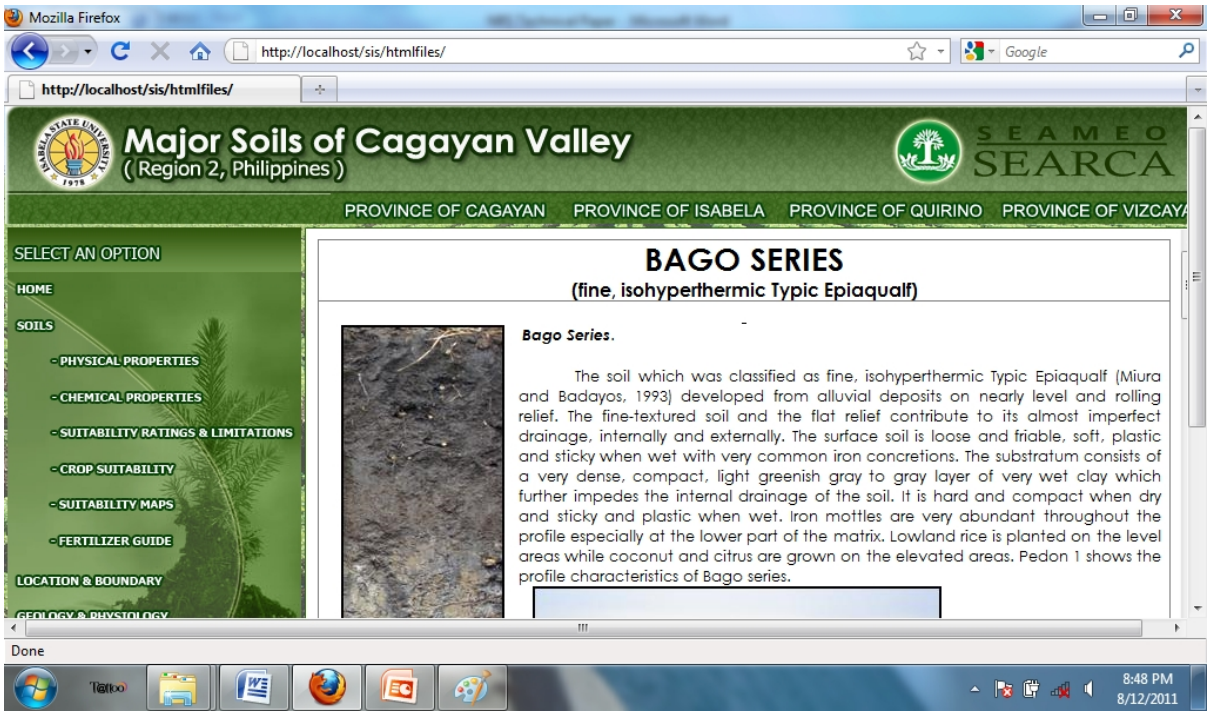


Lime and Fertilizer Guides of the Soils in Cagayan Valley



Specific Locations of each Soil Series
(Provincial, Municipal, Barangay)

Soil Series at Barangay Level



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VARIABILITY AND AGRONOMIC CHARACTERS OF ELITE LINES OF SOYBEAN (*Glycine max [L.]Merril*) from a Cross of 'Wilis' x B_{3570}

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ABSTRACT

High demand of soybean in Indonesia has caused this country to import the coomodity. Therefore, it is necessary for Indonesia to increase soybean production. The objectives of this study were (1) to look at the genetic variability of F_6 generations from a cross of 'Wilis' x B_{3570}, (2) look at agronomic characters of elite lines in F_7generation from a cross of 'Wilis' x B_{3570},. The study was conducted at the experimental station of The Faculty of Agriculture, The University of Lampung from April 2014 to April 2015. This study used a randomized complete block design with two replications with planting space of 20 x 50 cm. Results of the experiment showed (1) genetic variability in the F6 generation was narrow, (3) all genotypes tested has a greater mean of total seed weight per plant than that of the 'Wilis', B_{3570} and 'Gepak Kuning'

Keywords: *agronomic characters, soybean, variability.*

INTRODUCTION

According to BPS (2016), soybean production in Indonesia in 2015 was 963.099 thousand tons of dry beans, 0.85% higher than that in 2014. However, the increase has not met the national demand, so the country should import the commodity to meet the demand. One way to reduce imports is by increasing production using high-yielding cultivars which could be done through breeding. Soybean breeding to get the new varieties could be conducted through stages as follows, namely: (1) germplasm collection, evaluation, crossing, selection of crossbreds, yield trials, and variety release, or (2) the germplasm collection, evaluation with or without selection, test yield, and variety release (Kasno, 1992).

This study began with the selection of the existing collection, followed by crossing.A cross was made between varieties 'Wilis' and B_{3570}. 'Wilis' variety a have high yield, but is susceptible to a viral disease caused by soybean dwarf mosaic virus, while the B_{3570} is

reesistant to soybean mosaic virus but has a low yield (Barmawi, 2007) . In this study, yield and agronomic characters were evaluated and resistance to soybean mosaic viruswas not tested.

The results of the previous study showed that variability in all caharacters in the F_2 generation was broad except the characters the number of productive branches (Lindiana, 2012). In the F_3 generation genetic variability in the characters of time of harvest, number of productive branches, and a weight of 100 grains was narrow, while that for the characters of days to flowering, plant height, number of pods pithy, and seed weight per plant was broad (Wantini, 2013). In the F4 and F5 generation (Barmawi et al., 2013 and Meydina, 2014) genetic variability for all agronomic characters was found to be narrow.

In addition to variability, thepredictive value of heritability was also analyzed. The broad-sense heritability estimates on F_2 generation was found to be high for all the observed variables such as age of flowering, plant height, harvest time, the number of productive branches, number of pods per plant, seed weight per plant and weight of 100 grains. This seuggested that selection can be done in early generations to obtain expected genotypes that produce higher grain weight than the two parents. It was reported that selection in the F_2 generation resulted in 25 out of the 146 plants, which were better than the two parents (Lindiana, 2012). In the F_3 generation, 25 best individual plants out of 300 plants were better than the two parents (Wantini, 2013). In the F_4 generation,15 genotypes that were better than the two parents were selected (Barmawi, 2013). In F_5 generation, 26 out of 282 plants that are better than the two parents were selected (Meydina, 2014).

In the F7 generation of crossbred ‘Wilis’ x B_{3570}, the homozygosity rate was 98.43 % , so that phenotypes that appeared in the offspring will be relatively the same as the parents. Tests conducted on the performance of the agronomic characters would expectedly provide information on whether the characters of yield components would show a better performance than those of the parents.,The objectives of this study were (1) to look at the genetic variabilityof F_6 generation of crossbred ' Willis ' x B_{3570}. (2) to observe agronomic characters of several genotypes in F_7 generation of crossbred soybean ' Willis ' x B_{3570} .

MATERIALS AND METHODS

The study consisted of two experiments. The first experiment was conducted in April 2014 to July 2014 and the second one was held on December 2014 to April 2015. The experiments were conducted an experimental station, The Faculty of Agriculture, The University of

Lampung, Bandar Lampung, Indonsia. Materials used in the first experiment was 10 genotypes ofF_6 hybrid seed 'Wilis' x B_{3570}, 'Wilis', and B_{3570}, while in the second experiment was 11 genotypes ofF_7seed, 'Wilis', B_{3570}, and Gepak Kuning. Fertilizers used were urea 50 kg/ ha, TSP 100 kg/ha, KCl 100 kg/ha, the active ingredient Carbofuran Furadan, fungicidal active ingredient Mancozeb 80 %, active insecticide Delhtametrin 25 g/l and manure 10 ton/ha .

Both the first and second experiments were arranged in a randomized block design (RBD) with two replications with distance between genotype was 50 cm and the distance between plants in a row was 20 cm and there were 20 plants per experimental unit. Variancehomogeneitywas tested using Bartlett's test, and normality was tested using Tukeytest. If both assumptions were met then analysis of variance were done using random model (Baihaki, 2000). In the the first experiment, analysis of variance was calculated, in which the variability criteria was determinedon the basis of Anderson and Bancroft, 1952 cited by Wahdah, 1996. In the second experiment, superior genotypes which has middle value more than the comparison genotypes, based on advanced test LSI (Least Significance Increase) (Petersen, 1994) were selected.

Variables that were observed in the first experiment and the second were the day to flowering (HST), day to mature (HST), plant height (cm), the number of productive branches, number of pithy pods, 100-dry seed weight per plant (g), and dry seed weight per plant (g).

RESULTS AND DISCUSSION

The agronomic characters that showed narrow variability in the first experiment were presented in Table 1. This was because F_6 population had a high percentage of homozygote (98.44%), meaning that this population had narrow genetic variability. The results were in agreement with those reported by Meydina (2014) and Adriani (2014).Research conducted by Meydina (2014) showed that F_5 generation for all agronomic characters showed narrow genetic variability. Results of research on F_5 generations of crossbreeding Willis and Mlg $_{2521}$ conducted by Adriani (2014) showed that the genetic variability in characters flowering age, harvesting age, the number of productive branches, number of pithy pods, and seed weight per plant was narrow. Narrow genetic variabilityreported by Pinaria (1995) was due to the fact that the observed population belongs to the sixth generation of a cross.

A very high percentage of narrow genetic variability in th F6 generation suggests that all characters were located at homozygous loci (Table 1). A yield trial was carried out in

which 10 genotypes were grown, with 20 plants per genotype and with two replications, so that there were totally 400 plants. As many as 73 individual genotypes out of 400 plants had seed weight higher than the comparison genotypes. Eleven out of 73 genotypes were selected and grown for the second experiment to evaluate their agronomic characters.

All genotypes that had a lifespan of flowering and harvest time wider than the benchmarkwere tested in two experiments. Slower flowering genotypes showed a longer vegetative period so that more asimilates were expectedly stored and consequently they had havier seeds. Adie (2007) classifies the age of soybean in Indonesia into of five criteria, namely, very early maturing (less than 70 days), early maturing (70-80 days), medium (80-85 days), in (between 86-90 days), and very deep (over 90 days). Harvesting time was evaluated and it was found that the comparators had age belonging to very deep crop (> 90 days). Harvesting time was affected by the interaction between the environment and varieties (Sumpena et al., 2013). Under rainy condition, the age of the plant would be longer. This was what happed in the second experiment, which was conducted during the rainy season is in December 2014 to April 2015.

Higher plants of almost all genotypes tested were shorter than the comparison except genotype 142-159-1-14-1 that was higher than B_{3570} but shorter than 'Wilis' and Gepak Kuning (GK) (Table 2). On the basis of plant height, the ideal type (plant - ideotipe) of soybean plants should have height of 60-70 cm (Arsyad et al . , 2007). The genotipesin F7 in the study which did not have an ideal plant height is 142-159 genotypes (58.76 cm).

Number of branches of all genotypes was less when compared with GK, but when compared with 'Wilis', almost all genotypes had fewer number of branches except genotype 142-163-1-102. When compared with the B_{3570} , almost all genotypes had number of branches more than genotypes 142-163-1-1-2 and 142-163-1-1-14 (Table 2).

Number of pithy pods of all genotypes was less when compared with GK . When compared with Willis and B_{3570}, there were three genotypes that had less, namely genotype 142-163-1-1-2 , 142-159-1-16-12, and 142-159-1-14-1. This study supports research on F_6 populations showing that there were genotypes that had more than the number of pods of Willis and B_{3570} , whereas no inbreds had higher number of pods than GK pods .

Weight of 100 grains represent the size of soybean seeds. The seeds of all genotypes had larger size when compared with GK, whereas when compared with Willisand B_{3570} seed size, they were smaller except for genotype 142-159-1-14-1. Adie and Krisnawati (2007)

classifie the size of soybeans into 3 groups of seeds: small (< 10 g/100 grain), medium (10-14 g/100 grains), and large (> 14 g/100 grains). Based on these groupings, genotype 142-163-1-1-10 and 142-163-1-1-14 included in the criteria of being small seeds, while the other genotypes belonging to the character of large seeds. Large seed size is preferred by consumers, especially businessmen of tofu and tempeh (Adriani , 2014)

Grain weight per plant of almost all genotypes exceeded the 'Wilis'except genotype 142-163-1-1-2. When compared with almost all genotypes were hevier than B_{3570} except for genotype 142-163-1-1-2 and 142-159-1-16-12. When compared with GK, there are four genotypes that hada lower grain weight, i.e. genotipe142-163-1-1-2, 142-163-1-1-10, 142-163-1-1-14, and 142-159 -1-16-12. The remaining seven genotypes had seed weight per plant heavier than GK, Willis and B_{3570}.

Total weight of seeds per plant was the main variable that was observed because it was used as a reference for determining the productivity of crops. According to Basuki (2002), the physiological characteristics such as rate of photosynthesis, chlorophyll, the nitrogen content of leaves, pods and seed weight can be used as effective criterion in soybean yield improvement program. AnF_7generation wassubjected to selection based on the total weight of seeds and weight of 100 grains in orderto obtain genotypes of high productivity.

Based on the weight of seeds per plant and other characters such as the weight of 100 grains, the number of branches, number of pods and number of seeds per plant that were greater than the 'Wilis', B_{3570} and Gepak Kuning, the following genotypes have high potential to become high-yielding varieties, namely genotype 142 -102-4-6-4 ; 142-163-1-16-10 ; 142-159-1-16 -17 ; 142-159-1-16 -2 ; 142-159-5-1-6 ; 142-159-1-14-1 ; and 142-159-1-14 -12.

The genetic variabilityin F_6regeneration derived from a cross of 'Wilis' x $B_{3570}$was narrow. Genotype 142-102-4-6-4 , 142-163-1-16-10 , 142-159-1-16 -17 , -2 142-159-1-16 , 142-159-5- 1 -6 , 142-159-1-14-1, and 142-159-1-14 -12 was superior genotypes.

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Tabel 1. Genetic variability of F6 generation derived from a cross of ''Wilis'x B_{3570}.

Character	Genetic variability	σ_g	$2\sigma_g$	Criteria
Days to flowering	0,22	0,37	0,74	Narrow
Days to maturity	3,04	1,93	3,86	Narrow
Plant height	13,98	9,14	18,29	Narrow
Number of productive branches per plant	0,12	0,10	0,20	Narrow
Number of pods pithy	26,11	43,88	87,76	Narrow
100 seeds weight	0,28	0,16	0,31	Narrow
The weight of seeds per plant	3,50	3,92	7,83	Narrow

Genetic variability criteria were as follows :
$\sigma_g^2 > 2\ \sigma_{\sigma_g^2}$ extensive genetic variability
$\sigma_g^2 \leq 2\ \sigma_{\sigma_g^2}$: narrow genetic variability
(Anderson and Bancroft, 1952 in Wahdah, 1996

Tabel 2. Mean values of variables days to flowering and days to maturity with The comparative parents 'Wilis', B_{3570}, and Gepak Kuning.

No. Genotype	Days to Flowering				Days to Maturity			
	Mean	Comparison			Mean	Comparison		
		'Wilis'	B_{3570}	GK		'Wilis'	B_{3570}	GK
142-102-4-6-4	46,01	+	+	+	110,48	+	+	+
142-163-1-1-2	46,28	+	+	+	110,30	+	+	+
142-163-1-1-10	46,41	+	+	+	110,64	+	+	+
142-163-1-16-10	46,15	+	+	+	110,83	+	+	+
142-163-1-1-14	46,58	+	+	+	110,66	+	+	+
142-159-1-16 -17	45,99	+	+	+	110,36	+	+	+
142-159-1-16 -12	45,38	+	+	+	109,88	+	+	+
142-159-1-16 -2	45,86	+	+	+	110,47	+	+	+
142-159-5-1 -6	45,84	+	+	+	109,29	+	+	+
142-159-1-14-1	45,87	+	+	+	108,56	+	+	+
142-159-1-14 -12	46,06	+	+	+	109,98	+	+	+

(+) : longer than comparator atα = 5%
(-) : faster than comparator atα = 5%

3. Mean values of variables plant height and number of branches per Plantwith comparative parents 'Wilis', B_{3570}, and Gepak Kuning.

No. Genotype	Plant height				Number of branches per plant			
	Mean	Comparison			Mean	Comparison		
		'Wilis'	B_{3570}	GK		'Wilis'	B_{3570}	GK
142-102-4-6-4	68,61	-	-	-	9,82	-	+	-
142-163-1-1-2	67,25	-	-	-	9,06	-	-	-
142-163-1-1-10	70,79	-	-	-	10,06	-	+	-
142-163-1-16-10	61,00	-	-	-	9,94	-	+	-
142-163-1-1-14	63,99	-	-	-	9,21	-	-	-
142-159-1-16 -17	68,14	-	-	-	10,17	-	+	-
142-159-1-16 -12	58,76	-	-	-	9,87	-	+	-
142-159-1-16 -2	62,99	-	-	-	10,93	+	+	-
142-159-5-1 -6	64,89	-	-	-	10,21	-	+	-
142-159-1-14-1	69,84	-	+	-	10,38	-	+	-
142-159-1-14 -12	62,62	-	-	-	10,05	-	+	-

(+) : higherthan comparator at α = 5%
(-) : lower than comparator at α = 5%

Tabel 4. Mean values of variables number ofpithy pods, the weight of seeds per plant, and 100 seeds weightwith comparative parents 'Wilis', B_{3570}, and Gepak Kuning.

No. Genotype	Number of pods pithy				The weight of seeds per plant(g)				100 seeds weight(g)			
	Mean	Comparison			Mean	Comparison			Mean	Comparison		
		'Wilis'	B_{3570}	GK		'Wilis'	B_{3570}	GK		'Wilis'	B_{3570}	GK
142-102-4-6-4	14,74	+	+	-	65,18	+	+	+	14,13	-	-	+
142-163-1-1-2	13,59	-	-	-	56,77	-	-	-	14,38	-	-	+
142-163-1-1-10	14,65	+	+	-	61,99	+	+	-	13,58	-	-	+
142-163-1-16-10	14,56	+	+	-	64,16	+	+	+	14,57	-	-	+
142-163-1-1-14	14,92	+	+	-	62,87	+	+	-	13,54	-	-	+
142-159-1-16 -17	14,34	+	+	-	63,51	+	+	+	14,36	-	-	+
142-159-1-16 -12	13,94	-	-	-	58,33	+	-	-	14,28	-	-	+
142-159-1-16 -2	14,59	+	+	-	64,21	+	+	+	14,47	-	-	+
142-159-5-1 -6	14,77	+	+	-	65,69	+	+	+	14,59	-	-	+
142-159-1-14-1	13,98	-	-	-	64,66	+	+	+	16,23	+	+	+
142-159-1-14 -12	14,94	+	+	-	65,67	+	+	+	14,58	-	-	+

(+) : higher than comparator at α = 5%
(-) : lower than comparator at α = 5%

COMPARISON OF DIFFERENT MODELS IN ESTIMATING STANDARD EVAPOTRANSPIRATION IN LAMPUNG PROVINCE, INDONESIA

PURBA SANJAYA, TUMIAR K MANIK AND BUSTOMI ROSADI

ABSTRACT

Evapotranspiration (ET) is the loss of water to the atmosphere by the combined processes of evaporation from soil surfaces and transpiration from plants.Since various factors affect ET, including weather parameters; numerousequations have been developed to quantify standard ET. The equations vary in data requirements from very simple, empirically based or simplified equations to complex, more physically based equations. This study used six methods in estimating standard evapotranspiration using data from September 2011–Agustus 2012from Climate Station at Masgar (05°10'20" S, 105°10' 49"E, 50 m.a.s.l.) Lampung, Indonesia. The six models are: Hargreaves-Samani 1985 (H/S), FAO 24 *Radiation* (24RD), FAO 24 Blaney-Criddle (24BC), FAO 24 *Pan Evaporation* (24PAN), Linacre (Lina), andMakkink (Makk). The results were analyzed using statistics methods in error indicators, which are: *Root Mean Square Error*(RMSE), *Mean Absolute Error* (MAE), and *LogaritmicRoot Mean Square Error*(LOG RMSE), while the closeness among the models was analyzed using *Index Agreement* (I.A.). Direct measurement had also been done with measuring the water content inside lysimeters.The study concluded that Makkink model is the suitable simple model that should be chosen in Lampung lowland area to calculate $ET_o$when climate data is limited, besides the recommended FAO 56 Penman Monteith.

Keyword: Evapotranspiration, Standard Evapotranspiration, FAO 56 PM, Makkink Model

INTRODUCTION

Agriculture production in dry area is often limited by water availability. Two strategies to solvethe limited water availability areadjustingcrops planting dateto rainfall distribution and toirrigation schedule. Both strategies were based on crops water requirementestimated by evapotranspiration.

Evapotranspiration (ET) is the loss of water to the atmosphere by the combined processes of evaporation from soil and plant surfaces and transpiration from plants. Many factors affect ET, including weather parameters such as solar radiation, air temperature,

humidity,and wind speed; crop factors such as crop type, variety, density, and the stage of growth; and management and environmentalconditions such as soil conditions, salinity, fertility, crop disease, and pests (Allen *et al*. 1998). Therefore, an idea of reference (standar) evapotranspiration was developed.

Estimation of the evapotranspiration has been done since Penman (1948) derived the evapotranspiration formula based on the Dalton mass transfer equation and the energy balance equation. The effort started with estimating the reference evapotranspiration (ET_0) Reference ET is defined as ‘‘the rate of evapotranspiration from an extensive area of 0.08–0.15 m high, uniform, actively growing, green grass that completely shades the soil and is provided with unlimited water and nutrients’’ (Allen,*et al*., 1994 in Bakhtiari *et al*., 2011). More recently, Allen,*et al*. (1998) elaborated on the concept of ET_0, referring to an ideal 0.12 m high crop with a fixed surface resistance of 70 s m^{-1} and an albedo of 0.23.The surface condition should be met so that reference evapotranspiration only considered weather factors that influenced evapotranspiration rate.

Numerousequations have been developed to quantify potential evapotranspiration(PET). The equations vary in data requirements from very simple,empirically based or simplified equations requiring only monthly average air temperatures e.g., Thornthwaite (1948) and Blaney and Criddle (1950)andto complex, more physically based equationsrequiring, daily data for air temperatures, solar radiation, wind speed, and relative humidity e.g., FAO56-PM (Allen *et al*.,1998), as well as characteristics of the canopy surface e.g., Penman-Monteith (Monteith, 1965).

Actual ET for a specificcrop is called crop evapotraspiration (ETc). Since water availability is essential in agriculture production especially in dry areas, accurate and consistent estimates of crop evapotranspiration(ETc) in agriculture activities are important. The most common procedure for estimating ETc is to adjustthe reference evapotranspiration (ET_0) values with the crop coefficient (Kc); which$ETc=ET_0*Kc$. The Kc represents the integrated effectof changes in leaf area, plant height, irrigation method, rateof cropdevelopment, crop planting date, leaf area, canopy resistance,albedo, soil, climateconditions, and managementpractices (Doorenbos and Pruitt, 1977 in Irmak,*et al*., 2006).

The first step to calculate crops coeficient is by estimating the reference evapotranspiration. Reference evapotranspirationcan be measured directly by lysimeters; however, establishing and maintaining lysimeters for a long time period is costly, make it physically and economically impossible to measure evapotranspiration in every area of interest. Also, for a given vegetation type, potential evapotranspiration is a climatic parameter; so it can becomputed from weather data. Therefore, potential evapotranspiration could be estimated by theoretical orempirical equations, or derived simply by multiplying standardpan evaporation data by a coefficient.Equationsthat developed by Penman Monteith is the one recommended by FAO. However, since this equation needs various climate data, it is necessary to evaluate other available equations in case that the data is not available.

Lampung Province (103° 40' – 105° 50' E; and between: 6° 45' – 3° 45' S;35.288,35 km^2) is located at Southeast tip of Sumatra. Lampung climate is characterized by monsoonal rain distribution and local characteristics. Rain season in general is from October to March with the peak on January/February and dry season is from April to September. Monthly rainfall ranges from 50 – 200 mm and annual rainfall ranges from 1200 mm(lowland area) to 2500 mm (highland area). Lampung economic is dominated by agriculture products mainly coffee, chocolate, rubber and sugarcane. Lampung is also considered as main area for cash crops such as paddy, soybean and maize. Therefore, finding good and reliable method in estimating crops water requirement is necessary for better agriculture management.

The objective of this research were to compare different methods in estimating standard evapotranspiration for calculating crops evapotranspiration in Lampung area, Indonesia.

METHODS

This study used six methods in evaluating potentialevapotranspirationusing data from September 2011 to Agustus 2012from Climate Station at Masgar (05°10'20" S, 105°10' 49"E, 50 m.a.s.l.) Lampung, Indonesia.The six models are: Hargreaves-Samani 1985 (H/S), FAO 24 *Radiation* (24RD), FAO 24 Blaney-Criddle (24BC), FAO 24 *Pan Evaporation* (24PAN), Linacre (Lina), andMakkink (Makk). The results from those models were compared to FAO Penman-Monteith (56PM) as the standard model.To evaluate the relation betweenmodels, the results were analyzed using statistics methods in error indicators, which are: *RootMean Square Error*(RMSE), *Mean Absolute Error*(MAE), and*LogaritmicRoot Mean Square Error*(LOG RMSE), while the closeness among the models was analyzed using

Index Agreement (I.A.).Potential evapotranspiration was also observed in this study; using a lysimeter (3x2x1 m),a certain grass (*Sporabulusdiander*) which is the same type as timothy grass was planted on the common lysimeter. Since the lysimeter was maintained to have adequate soil water content, the evapotranspiration was evaluated by measuring the difference of soil water content every day. The measurements were done for 30 days using sensors called Kett gypsum block.

DESCRIPTION OF MODELS

1. Hargreaves-Samani 1985 (H/S) (Hargreaves and Samani, 1985)

The equation of this model is:

$$ET_o = 0.0023(T_{mean} + 17.8)(T_{max} - T_{min})^{0.5} R_a \quad (1)$$

With$ET_o$is standard evapotranspiration (mm/day), T_{mean} is daily mean temperature (^oC),$T_{max}$is maximum temperature(^oC), $T_{min}$is minimum temperature, andR_a Dailyextraterrestrial radiationof the atmosfer ($MJ/m^2/day$).

2. FAO 24 *Radiation* (24RD) (Doorenbos and Pruitt, 1977)

The equation of this model is:

$$ET_o = K_p \times E_{pan} \ldots\ldots (2)$$

$$K_p = 0.108 - 0.028u_2 + 0.0422\ln(FET) + 0.1434\ln(RH_{mean}) - 0.000631[\ln(FET)]^2 \ln(RH_{mean}) \ldots\ldots (3)$$

WithET_oisstandard evapotranspiration (mm/day), $K_p$ispan evaporation coefficient, E_{pan}isClass A pan evaporation (mm/day), $u_2$ismeanwind speed at 2m high (m/s), $RH_{mean}$is relative humidity (%), and FET is distance between pan and crops (m).

3. FAO 24 Blaney-Criddle (24BC) (Jensen, *et al*., 1990)

The equation for this model is:

$$ET_o = a + bf \ldots\ldots(4)$$

$$f = p(0.46T + 8.13).....(5)$$

$$a = 0.004RH_{min} - \frac{n}{N} - 1.41 \ldots\ldots(6)$$

$$b = 0.908 - 0.00483RH_{min} + 0.7949\frac{n}{N} + 0.768[ln(U_d + 1]^2$$
$$-0.0038RH_{min}\frac{n}{N} - 0.000443RH_{min}U_d + 0.281\left[ln(\frac{n}{N} + 1)\right] \ldots\ldots(7)$$
$$-0.0097[ln(U_d + 1][ln(RH_{min} + 1]^2\left[ln(\frac{n}{N} + 1)\right]$$

WithET_o is standard evapotranspiration (mm/day), P is percentage of day length, T is daily average temperature (˚C), RH is minimum relative humidity (%), n/N is ratio of possible actual day, and U_d is wind speed at 2 m (m/s)

4. FAO 24 *Pan Evaporation* (24PAN)(Doorenbos and Pruitt, 1977)

The equation of this model is

$$ET_o = K_p \times E_{pan} \ldots\ldots(8)$$

$$K_p = 0.108 - 0.028u_2 + 0.0422\ln(FET) + 0.1434\ln(RH_{mean})$$
$$- 0.000631[\ln(FET)]^2\ln(RH_{mean}) \ldots\ldots(9)$$

$ET_o$is standard evapotranspiration (mm/day), K_p is pan coefficient, $E_{pan}$is class A Pan evaporation (mm/day), $u_2$is average wind speedat 2m high (m/s), RH_{mean}relative humidity (%), andFET is distance between pan and green crops (m).

5. Linacre (LINA)(Linacre, 1977)

The equation of this model is:

$$ET_o = \frac{\left(\frac{500\,T_m}{100 - A}\right) + 15(T - T_d)}{(80 - T)} \ldots\ldots(10)$$

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$$T_m = T + 0.006h \ldots\ldots (11)$$

$ET_o$is standard evapotranspiration (mm/day),T is mean temperature (°C), $A$is latitude of the climate station (°), $T_m$is elevation of climate station (m), and $T_d$is average dew point temperature (°C). T_d equation is:

$$T_d = \left(\frac{f}{100}\right)^{\frac{1}{8}}(112 + 0.9T) + 0.1T - 112 \ldots\ldots (12)$$

$T_d$is average dew point temperature (°C), T is mean temperature (°C), and$f$ is average daily relative humidity (%).

6. Makkink (Makk) (Makkink, 1957).

The equation of this model is:

$$ET_o = 0.61\frac{\Delta}{\Delta + \gamma}\frac{R_s}{2.45} - 0.12 \ldots\ldots (13)$$

Which$R_s$is solar radiation (MJ/m^2/day), Δis vapor pressure curve (kPa/°C),andγis psychrometricconstant (kPa/°C).

7. FAO 56 PM (56PM) (Allen,*et al*., 1998)

The equation of this model is

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma\frac{900}{T+273}U_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34\,U_2)} \ldots\ldots (14)$$

ET_0 is standard evapotranspiration (mm/day), Rnis net radiation on crops surface (MJ/m^2/day),Giscontinuous heat flux to soil depth (MJ/m^2/day),Tis dailytemperature(°C),$U_2$is wind speed at 2 m (m/s),$e_s$is vapor pressure (kPa),$e_a$is actual vapor pressure (kPa),Δ is vapor pressure curve (kPa/°C),andγis psychrometric constant (kPa/°C).

In this study the ET_0 estimation fromFAO 56 Penman-Monteith model as the standard model was calculated using CROPWAT.CROPWAT isa computer program recommended by

FAObased onFAO 56 Penman-Monteithmodel (Allen,*et al*., 1998). Climate Parameters are needed by each model is presented in Table 1.

Indicators

The error indicators equation used to evaluate the model is:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(ET_{osi} - ET_{omi})^2} \dots\dots (22)$$

$$MAE = \frac{1}{N}\sum_{i=1}^{N}|ET_{osi} - ET_{omi}| \dots\dots (23)$$

$$LOG = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\log ET_{osi} - \log ET_{omi})^2} \dots\dots (24)$$

$$I.A = 1 - \frac{\sum(ET_{osi} - ET_{omi})^2}{\sum[|ET_{osi}'| + \|ET_{omi}'\|]^2} \dots\dots (25)$$

$$ET_{osi}' = ET_{osi} - \overline{ET_{omi}} \dots\dots (26)$$

$$ET_{omi}' = ET_{omi} - \overline{ET_{omi}} \dots\dots (27)$$

With$ET_{osi}$is Penman-Monteith standard evapotranspirationas the standard model, and$ET_{omi}$is others evapotranspirationmodels.

Table1. Climate parameters needed by each estimation model

No	Model	Climate data needed by each model							
		E_{pan}	T	R_s	R_n	RH	P	U_2	R_a
1	56PM		√	√	√	√	√	√	√
2	24BC		√			√	√	√	
3	H/S		√						√

4	Makk		√	√					
5	24RD		√	√		√		√	
6	24PAN	√				√		√	

RESULTS AND DISCUSSIONS

The first error indicator (RMSE) is presented in Table2. Based on the comparison among the six models, the error indicator RMSE ranged from 0.32-1.99 which means that ET_0 difference among the models was 0.32 mm to 1.99 mm/day. This is not a small number since 1 mm/day ET in 1 ha area is equivalent with water loss of 10,000 liter/day or 3.6 million liter/year.

Tabel.2. RMSE value among the estimating models of ET_0

	RMSE						
	56PM	Makk	24BC	24PAN	24RD	H/S	LINA
56PM	0	0,34	1,30	0,75	0,69	1,35	0,88
Makk	0,34	0	1,61	0,48	0,49	1,52	1,12
24BC	1,30	1,61	0	1,99	1,92	1,12	0,79
24PAN	0,75	0,48	1,99	0	0,33	1,93	1,54
24RD	0,69	0,49	1,92	0,33	0	1,98	1,54
H/S	1,35	1,52	1,12	1,93	1,98	0	0,59
LINA	0,88	1,12	0,79	1,54	1,54	0,59	0

Using Lampung climate data, the lowest RMSE was found betweenFAO 24 *Radiation*andFAO 24 *Pan Evaporation*while the highest RMSE was found between model FAO 24 *Pan Evaporation*andFAO 24 Blaney-Criddle. For Lampung, estimation ET model with the closest estimation to FAO 56 Penman-Monteith is Makkink model with RMSE value 0.34.

The second error indicator (MAE) is presented in Table 3. Similar results with RMSE were found in error indicators bothMAE and log RMSE (Table 4). Makkinkmodel was the model which is closest to FAO 56 Penman-Monteith.

Table.3. MAEvalue among the estimating models of ET_0

	MAE						
	56PM	Makk	24BC	24PAN	24RD	H/S	LINA
56PM	0	0,28	1,06	0,62	0,67	1,28	0,86
Makk	0,28	0	1,28	0,40	0,45	1,50	1,08
24BC	1,06	1,28	0	1,69	1,74	0,95	0,68
24PAN	0,62	0,40	1,69	0	0,25	1,90	1,48
24RD	0,67	0,45	1,74	0,25	0	1,95	1,53
H/S	1,28	1,50	0,95	1,90	1,95	0	0,47
LINA	0,86	1,08	0,68	1,48	1,53	0,47	0

Tabel.4.LOG RMSE among the estimating models of ET_0

	LOG RMSE						
	56PM	Makk	24BC	24PAN	24RD	H/S	LINA
56PM	0	0,04	0,13	0,11	0,10	0,14	0,10
Makk	0,04	0	0,17	0,08	0,07	0,16	0,13
24BC	0,13	0,17	0	0,23	0,22	0,11	0,08
24PAN	0,11	0,08	0,23	0	0,06	0,23	0,20
24RD	0,10	0,07	0,22	0,06	0	0,23	0,19
H/S	0,14	0,16	0,11	0,23	0,23	0	0,06
LINA	0,10	0,13	0,08	0,20	0,19	0,06	0

MAE between FAO 56 Penman-Monteith and other modelsranges from 0.28 mm/day (Makkink) to 1.28 mm/day (Hargreaves-Samani 1985) and LOG RMSE ranges from 0.04 mm/day (Makkink) to 0.11mm/day (FAO 24Blanney-Criddle).

Table 5 shows the results of Index of Agreement (I.A.). Consistently, Makkink model gave the best results with I.A. 0.77 followed by Linarch (0.42) and FAO Pan evaporation (0.42)

Tabel.5.Index of Agreement among the models

	I.A						
	PM	MK	BC	Pan	24 RD	HS	Linarch
PM	1	0,78	0,09	0,42	0,55	0,26	0,42
MK	0,78	1	-0,35	0,80	0,81	0,10	0,10
BC	0,09	-0,35	1	-0,40	-0,22	0,79	0,85
Pan	0,42	0,80	-0,40	1	0,95	-0,03	-0,08
24 RD	0,55	0,81	-0,22	0,95	1	-0,03	-0,01
HS	0,26	0,10	0,79	-0,03	-0,03	1	0,93
Ln	0,42	0,10	0,85	-0,08	-0,01	0,93	1

From those results, it can be concluded that Makkink model is the suitable simple model that should be chosen in Lampung to calculate ET_o besides the recommended one FAO 56 Penman Monteith, especially when the climate data is limited.

So far the estimating model that broadly used is FAO 24 PAN which is based on observation on class A evaporation pan. This model did not give a good estimation compared to the FAO 56 PM model (RMSE 0.75; MAE 0.62; Log RMSE 0.11 and I.A. 0.42). In comparing 24 PAN model to 56PM, using 3 years data in 2 stations in Lampung, Manik*et.al.*(2012) found that the coefficient correlation between those two models are low (r=0.3 for Branti Station and 0.5 for Masgar station).

Monthly average ET_0 results from each model in 1 year is presented in Figure 1. Most of the models had the similar trends with FAO 56 PM but with different closeness. Some models underestimated FAO 56 PM (Makk, FAO 24 RD and FAO 24 PAN) while some overestimated (24 BC, H/S and Lina). Makkink(Makk) model was closely similar with FAO

56 PM in month of January-March, October -December) and a little underestimated in March – October (dry season).

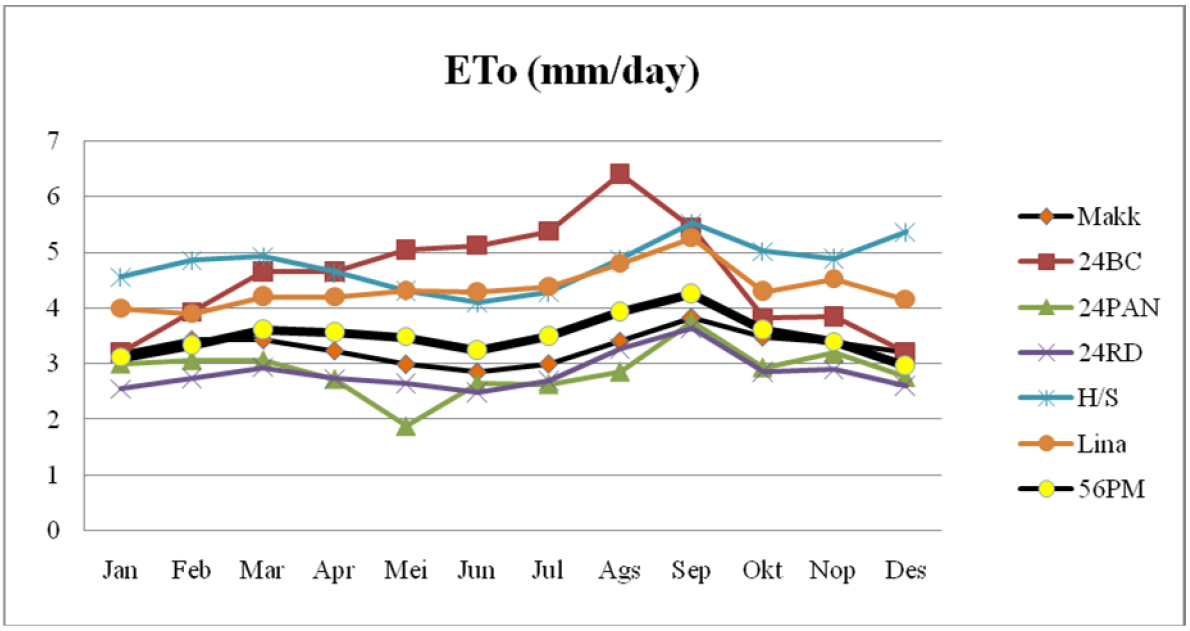

Figure 1. Monthly average of ET_o results from each model in 1 year

Research about comparing different models has been done in some countries. *Chen et al* (2005) used 7 estimating models in four provinces of Taiwan and found that Makkink and Hargreaves-Samani models were the best models in estimating ET_o when compared to FAO 56 PM. Chowhury,*et al* (2010) also found that in India, Makkinkmodel had the closest estimation to FAO 56 PM with a little underestimated result.

Makkink model (equation no. 13) is the simplest model among others in this study, the model is calculated use only two basic data, maximum and minimum temperature data. Psychrometricconstantis 66,1 kPa/^oC, R_s and Δ are calculated by these equations below.

$$R_s = \sqrt{(T_{max} - T_{min})}R_a \ldots\ldots (28)$$

$$\Delta = \frac{4098\left[0{,}6108\, exp\left(\frac{17{,}27\,T}{T+237{,}3}\right)\right]}{(T+237{,}3)^2} \ldots\ldots (29)$$

Which Rs is solar radiation (MJ/m2/day), Δ is vapor pressure curve (kPa/^{o}C), and Ra is daily extraterrestrial radiation of the atmosfer (MJ/m2/day) from FAO Irrigation and drainage paper 56meteorological data table (Allen,*et al*., 1998).

Xu and Chen (2005) did similar study in Germany with comparing 7 models and found that Granger-Gray and Makkink models were the best models for the area. In North China Schneider,*et al*. (2007) compared 4 models with direct observation and concluded that Hargreaves-Samani and Makkink models were the best models in estimating ET_o even better than FAO 56 PM.

Jacobs,*et*al (2004) conducted research on estimating ET_0 in Florida, using remote sensing method with data from GOES. The results showed that FAO 56 PM is the best model with R^2= 0.92 however this result is not much different with estimated results from Makkink model which gave R^2= 0.90.

The results for direct measurement inside lysimeters(Table 6.) show that evapotranspiration rate predicted by models were higher than measured by gypsum block. Potential evapotranspiration calculated by models show the atmosphere power in evaporating water on soil surface; the rate should be higher since in Tropical area because the radiation is intense and air temperature generally high. Soil moisture reflects the balance of precipitation, runoff, ET and exhibited various types of pulse events (*Wang,et al*., 2012). Because of soil water movement, on soil surface water availability could be limited even when it kept being watered because the water percolates to the deeper level but it also could be moist even when no water added to it since water could be moves up to the surface . When atmosphere power to evaporate water is higher than soil moisture, it could be concluded that during the observation the soil surface was not wet enough to meet atmosphere needs.

Table 6. Actual Evapotranspiration observed by soil water content method and compared to potential evapotranspiration by FAO 56 and Makkink models

Day of Observation	Soil Tension (kΩ)	Volumetric Soil water content(%)	Δ S (%)	Cummulative Water loss (mm)	ET (mm)	ET FAO 56 (mm)	ET Makkink (mm)
1	122	43.963			0.000	2.67	3.11
2	113	45.044	2.701	2.701	0.000	4.04	3.64
3	131	42.960	-5.210	-2.509	5.210	2.52	2.85
4	143.33	41.691	-3.171	-5.680	3.171	4.34	3.63
5	146.67	41.367	-0.812	-6.492	0.812	4.94	3.54
6	157.33	40.377	-2.473	-8.965	2.473	4.4	3.44
7	160	40.140	-0.593	-9.558	0.593	2.17	3.25
8	154	40.679	1.347	-8.211	0.000	4.49	3.48
9	153.67	40.709	0.076	-8.135	0.000	2.41	2.76
10	152.67	40.801	0.230	-7.905	0.000	3.94	3.56
11	148.33	41.208	1.017	-6.889	0.000	3.78	3.26
12	169	39.368	-4.599	-11.487	4.599	4.59	3.44
13	171	39.203	-0.415	-11.902	0.415	2.98	3.02
14	168.33	39.424	0.555	-11.347	0.000	3.09	3.43
15	179	38.558	-2.166	-13.514	2.166	2.72	2.9
16	176.67	38.743	0.462	-13.052	0.000	3.73	3.19
17	188.33	37.841	-2.253	-15.305	2.253	3.18	2.58
18	203.33	36.761	-2.701	-18.006	2.701	2.93	2.97
19	236.67	34.620	-5.352	-23.358	5.352	3.19	2.87
20	280	32.249	-5.926	-29.285	5.926	3.57	3.13
21	246.67	34.036	4.468	-24.817	0.000	4.5	2.8
22	249	33.904	-0.331	-25.148	0.331	4.14	3.61
23	251.67	33.754	-0.376	-25.524	0.376	2.32	2.78
			Total ET (mm)		36.380	77.970	70.130
			Average ET (mm)		2.599	3.506	3.184

This study concluded that Makkink model is a simple model that can be chosen in Lampung as an alternative to calculate standard evapotranspiration in an area with limited climate data needed to apply FAO 56 PM. During the study, water availability on soil surface was not enough to meet atmosphere need; this condition could limit crops growth.

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ENHANCED RESISTANCE OF TOMATO PLANTS TO *Fusarium* sp. BY TREATING SEEDS WITH A 0.2 mT MAGNETIC FIELD

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ABSTRACT

Treatment of tomato plants with a magnetic field is known to increase vigor and yield. In this study, we examined iftreatment of tomato seeds with a magnetic field of 0.2 mT could enhance tomato plantsresistance to *Fusarium*sp, a fungus that cause wilt disease in tomato plants.The research was conducted in a split-split-plot design. Treatments were arranged in factorial, with length of exposure to 0.2 mT magnetic field (M) as the main plot consisted of: control, 7'48 "; 11'42" and 15'36 "; methods of inoculation with *Fusarium*sp. (F) as subplot; and soaking the seeds in water before treatment (S) as the sub-sub plot. The sub plot consisted of two methods of inoculation with *Fusarium*(F), i.e. through seeds before seed sowing and through the stem when the plants was at 28-days after seed sowing (das). The sub-sub-plot consisted of two treatments, i.e., seed soaking in water (S) for 15 minutes prior to treatment with magnetic fields and as the control treatment, tomato seeds weredirectly exposed to magnetic fields, without soaking in water. Parameters measured were: plant dry weight at 42 das; number of flowers at the beginning of flowering; number of fruits, fruit fresh weight, and fruit diameter.The results showed that treatmentsof tomato seeds with 0.2 mT magnetic field (M) improvedall of plant growth and yield parameters, even after the tomato seedswere inoculated with*Fusarium* sp., with the exceptionin fruit diameter. There was no interaction between the seed exposure to magnetic field and seed soaking in water (M x S). In addition, methods of inoculation with *Fusarium* sp.resulted in significant difference in fruit diameter. Soaking of the seeds in water before exposure to magnetic fields increasedplant dry weight, and number of flowers, but a decrease in fruit diameter.

Keywords: magnetic field, methods of inoculation, seed soaking in water, *Fusarium*sp, growth and yield.

INTRODUCTIONS

Promising prospects of the magnetic field (MF) energy utilization to improve the quality and agricultural field production have been documented from various studies.In soybeans, the use of a MFof 0.1 mT for 15 min 36 sec increases the activity of α-amylase and accelerate the germination of white soybean, while an increase in the activity of α-amylase and germinationspeed in green beans were obtained from the seeds exposed to a MF of 0.1 mT for 11 min 44 sec and 15 min 36 sec (Anggraini *et al*., 2013,and Anggraini *et al*., 2013).Atak *et al*. (2007) showed that a treatment of MF 2.9 to 4.6 mT for 2.2 to 9.8 seconds increased the formation of buds, roots, chlorophyll, peroxidase enzyme activity in cultured soybean cotyledons. The increase in germination capacity, decrease in number of diseased seedlings and deformed seedlings as a result of MF treatment was also shown from thelow quality carrot seeds due to infection by the fungus *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko and *A. radicina* Meier, Drechsler & E.D. Eddy that cause rot sprouts (Dorna *et al*., 2010).

The positive impact of MF treatment in plants has also been demonstrated on the seeds of green onion (Novitsky *et al*., 2001), strawberry (Esitken and Turan, 2004), tobacco (Aladjadjan, and Ylieve. 2003), and cotton (Nagy *et al*., 2005). Previous research on tomato proved that the exposure of the dynamic MF of 120 mT for 10 min and 80 mT for 5 min improved all parameters of vegetative and reproductive growth (De Souza *et al*., 2005). The same results on tomato plants were also obtained from MF treatment 0,2mT for 7 min 48 sec. However, this treatment did not increase the percentage of germination and seedling growth rate (Agustrina *et al*., 2012). Soaking the seeds of tomatoes prior to a treatment with MF of 0.2 mT increased the size of the stomata, parenchymal cells, and the bundle cell diameter (Agustrina *et al*., 2011).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops economically, because it can be used both as a vegetable and raw materials for various food industry. Tomato production is often constrained by pathogens including *Fusariumoxysporum*, that causes *Fusarium*wilt disease (Smith *et al.*, 1988). Ignjatov *et al*. (2012) explained that *Fusarium*is the causal agent of tomato wilt disease that causes root and basal stem deterioration and results in the wilting ofvegetable plants. In addition, browning of the vascular tissues is a strong evidence of *Fusarium* wilt. In this study, we examined

whether an exposure of tomato seeds to magnetic fields influenced growth and yield, or followed by an increase in resistance of tomato to disease caused by *Fusarium* sp .

MATERIALS AND METHODS

Tomato seeds were obtained from seed market with a germination rate of 95 %. Immerssion treatment (S1) was conducted by immersing the seeds in tap water for 15 minutes before being exposed to a 0.2 mT magnetic field (MF) treatments (M), while the untreated seeds (S0) were directly exposed to the MF without immersion in water. The MF treatments were given infour different levels of exposure time: 7 min 48 sec (M1); 11min 42sec (M2) and 15min 36 sec (M3) and control (M0).

A *Fusarium* suspension containing monospores at a density of $1x10^7$derived from isolate of *Fusarium* sp. provided by Bogor Agriculure University (IPB) culture collections wasused for *Fusarium*inoculationtreatments (F). The inoculation methods consisted of inoculation through the seeds and through the stem. Inoculation through the seeds was done by soaking the MF-treated seedsin the suspension containing monospores of *Fusarium* sp. for 12 hours, while inoculationthrough the stem was performed by injecting about 50 ml suspension of *Fusarium*monosporesto tomato stems at the age of 28 days after sowing (DAS). All of the seeds inoculatedwere then planted in either sterile or non-sterile soil. The marking for the treatments of *Fusarium* inoculationwere as follows. F0 = uninoculated-seeds grown in sterile soil, F1 = uninoculated-seeds grown in non-sterile soil, F2 = inoculated-seeds grown in srerile soil, F3 = inoculated-seeds grown in non-sterile soil, F4 = stem-inoculated plantsgrown in sterile soil, and F5 =stem-inoculated plants grown in non-sterile soil.

The procedures for planting and maintenance of the tomato plants followed those as usually done by farmers.The plant responses to the treatments of MF, to inoculation with *Fusarium*monospores and to seed soaking was observed by measuring dry weight of plants at 42 DAS, the amount of flowers at the beginning of flowering, the number of fruits, the fresh weight of fruits, and fruit diameter. Data were subjected to analysis of variance, followed by mean separations using least significant difference (LSD) at α = 5% (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Results of analysis of variance at α = 5% showed that both exposure of tomato seeds to MF (M) and the interaction of MF vs. seed soaking before the MF treatments (M x S)

significantly affected all parameters measured (Fig. 1-5). However, the inoculation treatment with *Fusarium* sp. (F), its interaction with the MF exposure (F x M), its interaction with seed soaking before MF treatments (F x S), as well as interactionsbetween the MF exposure, *Fusarium*inoculation, and seed soaking before MF treatments (M x F x S) did not affecttomato plant growth and yield significantly.Previous results (Dorna *et al*., 2010) using poor quality seeds of carrot cv. 'Nantejska' and 'Perfekcja' due to fungal infection, showed that seed exposure to MF could improved the germination capacity, although did not significantly affect the cv. 'Perfekcja'. In addition, the seed exposure to MF also decreased the number of diseased seedlings in both cultivars. Dorna *et al*. (2010) suggested that the MF treatmentsto some extentcould control the growth of patogenic fungi in the seeds, which in turned decrease both the number of diseased seedlings and dead seeds. El Nabi *et al*. (2013) proved that exposure to the dynamic MF of 7-15 gauss decreasedthe disease severity as well as the disease incidence on onion seedlings. The magnitude of the power dynamic MF given did not give different results but increasing the exposure time of dynamic MF further reduced the severity and the onset of the disease.Other reports in the effects of MF treatments on the growth of some pathogenic fungi (Nagy, 2005) showed that the use of MF at 0.1, 0.5 and 1 mT decreased the growth ofcolonies by 10%. At the same time, the number of *Fusarium oxysporum* conidia decreased by 79-83 %. However, the number of the developed conidia of *Alternaria alternata* and *Curvularia inaequalis* increased by 68-133 percent.

In this experiment, the MF treatments significantly increased the dry weight of tomato plants measured at 42 days after seeding (Figure 2a), the number of flowers (Figure 3a), the number of fruits (Figure 4a), and the fruit fresh weight (Figure 5a). The results obtained in this study were consistent with those from previous research (De Souza *et a*l., 2005), which showed that treatments with MF improvedthe dry weight of roots, stems, and leaves of tomato plants, as well as flower numbers, fruit numbers and fruit fresh weight compared to the control treatment.In addition, there was no significant difference in all measured parameters in treatment of exposure with 120 mT MT for 10 min compared to the exposure with 80 mT MT for 5 min.

Increased in plant dry weight of tomatoes plant from these results are similar to the previous results showing that exposure to MF 0.2 mT for 7 min 48 sec increase the rate of growth of tomato plants, the fresh weight and dry weight, as well as plant leaf area measured at 60 days after planting (Agustrina *et al*., 2012). De Souza *et al*. (2005) found that increase

in dry weight of tomato plants was a positive result of the initial effects of MF since they appeared to induce an improved capacity for nutrient and water uptake, providing greater physical support to the developing shoot which were also observedfrom the results of their research. The increase in root length as a result of MF treatment was also observed on lettuce seed germination (Mousavizadeh *et al*., 2013 and Soltani and Kashi, 2004).

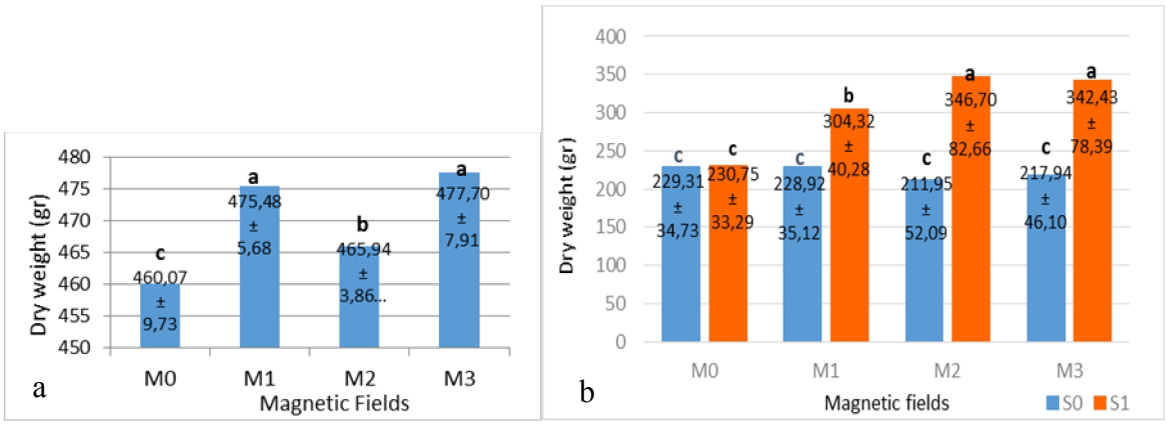

Figure 1. Dry weight of tomato plants at 42 days after seedling (DAS) under MF treatments (a) and treatment interaction of MF and seed soaking before MF (b).

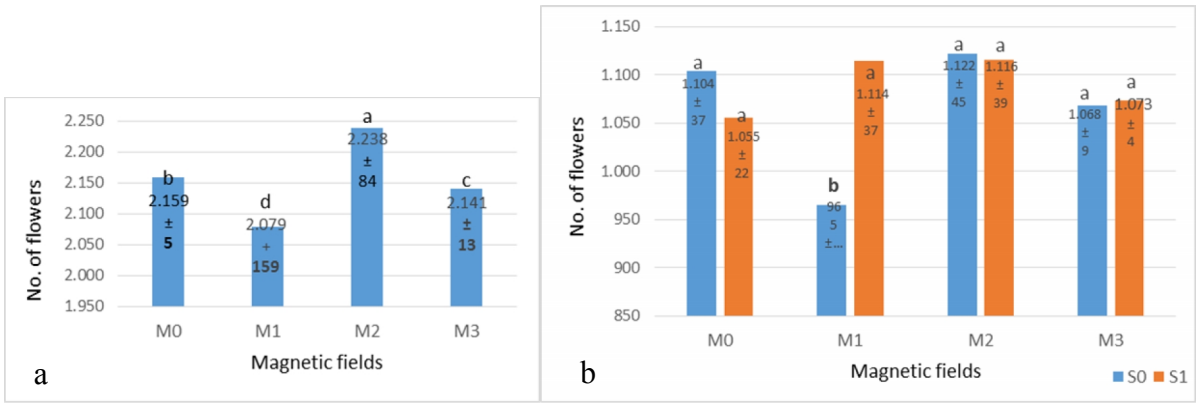

Figure 2. Number of flowers of tomato plants under MF treatments (a) and treatment interaction of MF and seed soaking before MF (b). Tomato seeds without MF treatments (M0), exposure to MF for 7 min 48 sec (M1); 11min 42sec (M2) and 15min 36 sec (M3). Tomato seeds were not soaked (S0) and soaked (S1) before MFtreatments. The same letter within the figure indicates the lack of a significant difference ($p < 0.05$) according to LSD test.

Dorna *et al*.(2010) and (Reina*et al*., 2001) explained that he influence of a magnetic field on seeds might be a short-term, e.g. 10 min, followed bythe effect in the form of increased water

absorption occurring after several hours and lasting for at least several days. This presssumption is convinced by the variant, in which an effectiveaction of a MF on plants, including germination, may be replaced by the MF exposure on water or substrate which is used to water plants (Aboe El-Yazied*et al.,* 2011; Hozayn*et al*., 2010; and *Morejon et al., 2007*).Water is an important factor for the metabolic processes in the cells. The magnetic field affects the physical and chemical properties of water by lowering the surface tension and increases the viscosity of the water so it is more stable with lower molecular energy and higher activation energy Cai *et al*. (2009). The studies on water exposed to the action of a magnetic field indicate an altered, usually reduced, surface tension, viscosity (Pang and Deng, 2008). Based on several other studiesMorejon *et al.* (2007) explain that magnetic field exposure to normal water (water+ions) changes some of its physical and chemical properties such as: surface tension, conductivity, solubility of salts, refractive index and pH. By application of magnetically treated water(MTW) in pine seed germination, he showed that it improves germination percentage. These results leads to a hypothesis that MTW properties as mentioned above make it much easier to penetrate inner parts of the seed. Several other theories have also been proposed to explain the mechanism of MF effect in plant germination metabolisma, includingbiochemical changes or altered enzyme activities(Majd and Shabrangi, 2007) such as amylase (Angrainie*t al*., 2013, Rochalska and Grabowska, 2007) protease, lipase (Rajendra *et al*., 2005).Then an increase in vigor as a result of MF treatment is thought to have an important role in inducing subsequent metabolism necessary for plant growth and development so that the plant reached the phase of vegetative and reproductive.

Atak *et al*. (2007) proved that MF exposure increases chlorophyll content. Exposure magnetic field also increases the size of the stomata (Setyasih *et al.,* 2013), and the surface area of leaves (Agustrina *et al*., 2012 and De Souza *et al*., 2005). All the process parameters of growth are inextricably linked to each other so that eventually resulted in higher dry weight (Figure 1a).This study also showed soaking seeds MF exposure increases dry weight (Figure 2b), which reinforced the results of previous research on tomato (De Souza *et al*., 2005)Data from this research and previous studies reinforce the confidence in the essential evidence of the effect of the MF exposure to increase in the rate of photosynthesis.Sources of energy produced from photosynthesis in the late vegetative stage should be sufficient to support the processes of development and differentiation plant to enter the phase metabolic

reproduction, which is the very complex process of metabolism and requires a lot of energy, among others in the process of establishing tissue cells needed to form reproductive organs.

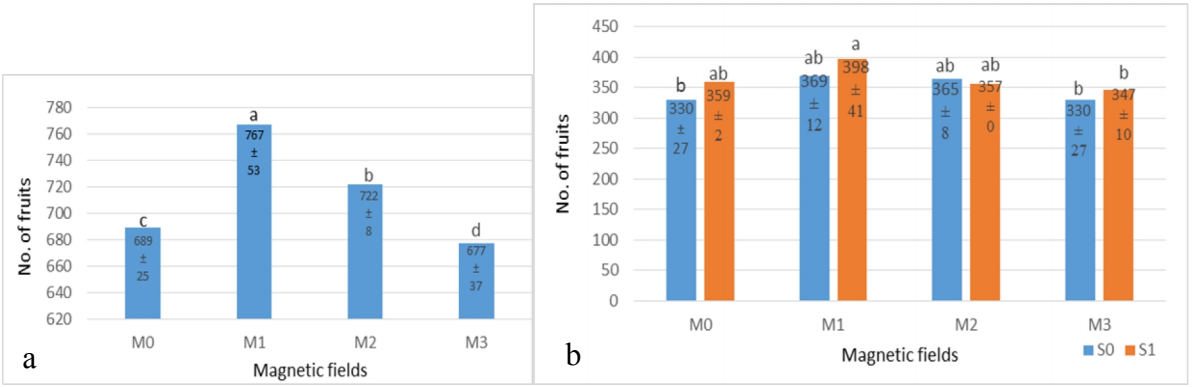

Figure 3. Number of fruits of tomato plants under MF trealment (a) and treatment interaction of MF and soaking seed before MF (b).

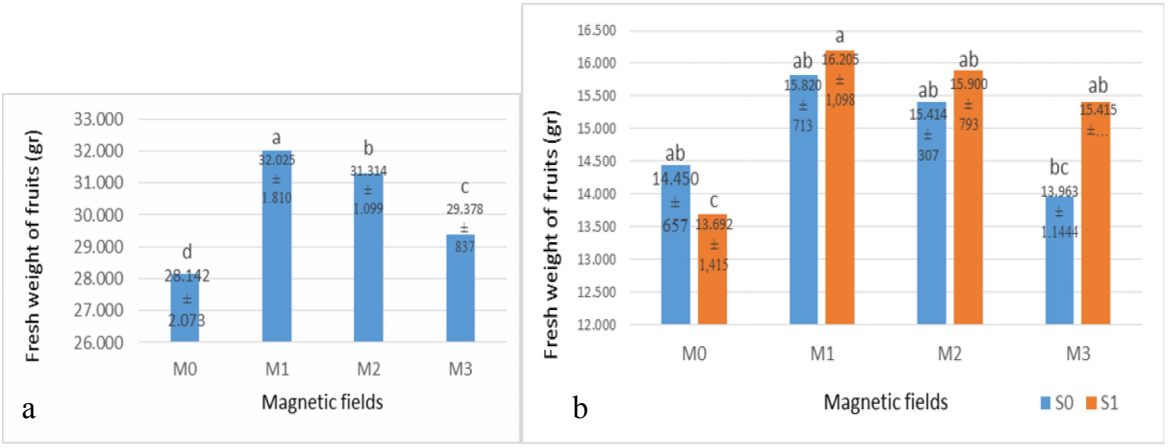

Figure 4. Fresh weight of fruits of tomato plants at 42 das under MF trealment (a) and treatment interaction of MF and soaking seed before MF (b).

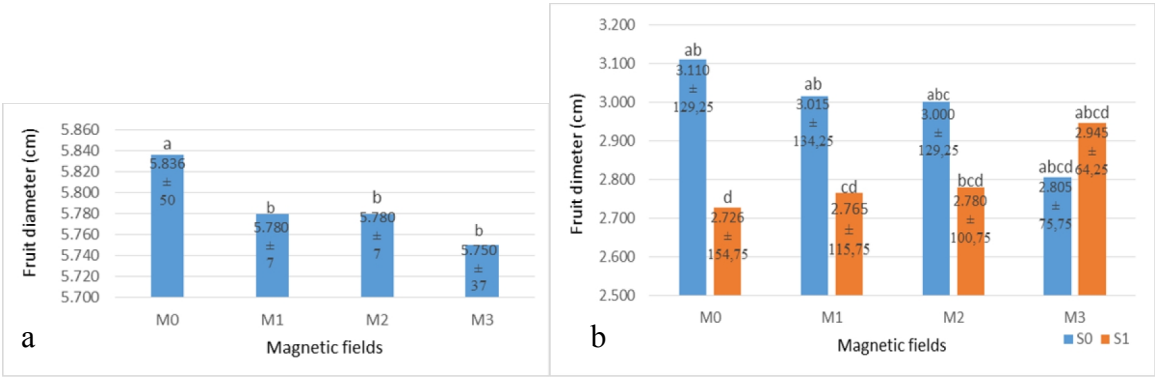

Figure 5. Fruit diameter of tomato plants at 42 das under MF trealment (a) and treatment interaction of MF and soaking seed before MF (b). Tomato seeds without MF treatmen (M0), exposure to MF for 7 min 48 sec (M1); 11min 42sec (M2) and 15min 36 sec (M3). Tomato seeds were not soaked (S0) and soaked (S1) before MFtreatments. The same letter within the figure indicates the lack of a significant difference ($p < 0.05$) according to LSD test.

The data in Figure 1a and Figure 2a shows that in the treatment of MF which resulted in high dry weight, forming the fewnumber of flowers. In this study, dry weight measurements were carried out at the same time calculating the number of flowers, when the plant has entered the generative phase.It seems that, plants that produce more flowers, spent more energy source of photosynthesis, so the remaining energy sources became less (Fig 2a).The initiation of flowering buds implied a fundamental changes in developmental programme, as indicated by the large change in RGR from a two-compartment systems (shoot and root) to a three-compartment systems (shoot, root and reproductive structures). Leaf massfraction (LMF, fraction of total resources invested in leaves) declined during the reproductive stage.But when calculated as fraction of the total vegetative resources it stayed the same (KoelewijnKoelewijn, 2004).

Figure 6. A healthy tomato plants (S0F3M2-1 and S1F2M1-2) and tomato plants infected by *Fusarium* wilt disease (S0F5M3-2 and S1F1M0-2).

The results on the number of fruits, fruit weight, and fruit size did not indicate a consistent response to the treatment of MF and the seedsoaking before being exposed to a MF. The MF treatment that produce the highest number of flowers was not followed by the highest results of the fruit numbers. These results are thought to be related to the influence of *Fusarium* sp. inoculation through seeds or stems in this study, although they did not resulted in significant effects.Figure 6 shows that plants were infected by *Fusarium* although they could survive and produced fruits. Steinkellner *et al*. (2005) explained when symptoms due to *Fusarium* sp infection occurred at the beginning of growth, this led to the death of the plant, but when symptoms due to *Fusarium* sp infection occurred after the plant in mature phase, the plants could still survive and grew, although the yield would decline. It was suggested that an increase in the activity of enzymes peroksidase on plants from seeds exposed to a

magnetic field led to tomato plants survived from *Fusarium* spinfection(Atack *et al*, 2005).Ohja and Hatterjee (2012) found higher peroxidase activities in *Fusarium oxysporum*-infectedtomato leaves than those in healthy leaves, and the enzyme activities increased with increasing duration of inoculating the leaves with the fungus.Peroxidases (POX) are heme-containing glycoproteins and suggested to have important roles in lignification, suberization,and self-defense against pathogens (Hiraga *et al*., 2001).

In this study, it was is also found that soaking seeds before MF exposure increased the fruit fresh weight (Figure 4b), but did not increase fruit size (diameter) (Figurae 5b). These resultswere probably because the weight of the fruits measured was the total weight of the fresh fruits produced by the plants. Therefore, plants with more number of fruits would produce fruits with smaller sizes than those that produced less fruit numbers.

Exposure tomato seeds to MF might have enhancedthe resistance of tomato plants against *Fusarium oxysporum* infection, since the seed soaking prior to MF exposure increased plant dry weight. However, the response of the reproductive parameters to the seed soaking treatments before MF exposure wereinconsistent despite showing significant effects.

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MACROALGAE (*Sargassum sp., Gracillaria sp.*) AND TAURINE ON DECREASE THE TOTAL CHOLESTEROL LEVEL OF HYPERCHOLESTEROLEMIA MALE MICE (*Mus musculus* L.)

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ABSTRACT

This study aims to determine the ability of *Sargassum* sp., *Gracillaria* sp, and taurine as well as the most effective treatment in reducing total cholesterol level of hypercholesterolemia of male mice (*Mus musculus* L). Mice were divided into 8 treatment groups with 5 replications, they were group C- given standard diet and group C+ fed with suspension of cow brain as hypercholesterol diet of 1 ml/day until the 28thday, CC, T1, T2, T3, T4 and T5 were fed with hypercholesterol diet for 14 days, and then at the 15th day group CC were fed with standard diet, T1 were fed with 15,6 mg/gmBW of taurine, T2 were fed with 32 mg/gmBW of *Sargassum* sp., T3 were fed with mix of 32 mg/gmBW of *Sargassum* sp. and 15,6 mg/gmBW of taurine, T4 were fed with 32 mg/gmBW of *Gracillaria* sp. and T5 were fed with mix of 32 mg/gmBW of *Gracillaria* sp. and 15,6 mg/gmBW of taurine until the 28th day. The results showed that the highest decrease of total cholesterol levels was in group T5 reaching 93,8 mg/dL in 14 days. The average of total cholesterol levels on groups T2, T3, T4, and T5 were not significantly lower than group contol (CC) (P>0,05).

Keywords: Taurine, *Sargassum* sp., *Gracillaria* sp., total cholesterol level, hypercholesterolemia.

INTRODUCTION

Hypercholesterolemia was defined as a disease caused by excess levels of cholesterol in the blood which mainly caused by high intake of food containing fat. The higher level of cholesterol leads to atherosclerosis affecting coronary heart disease and stroke (Kaplan and Stamler, 1994). Most of case, hypercholesterolemia was treated using synthetic drugs. These synthetic drugs, although effective, they show some side effects as well high price (Hicow, 2011). Therefore, it is necessary to elaborate some natural cure for hypercholesterolemia which provide priceless and no side effect.

Sargassum sp., seaweed from brown algae, are known to lower blood cholesterol levels with its active compound and high fibres. Some study indicated that the content of sodium alginate in *Sargassum filipendula* can lower total blood cholesterol levels of male rats conditioned with hypercholesterolemia (Wikanta *et al.,* 2003). Besides *Sargassum* sp., *Gracillaria* sp was also known to reduce cholesterol with its high fibres content such as gelatin and carrageen. *Gracillaria* sp. belongs to red algae (Rhodophyta). The active compounds of *Gracillaria* macroalgae that play a role in lowering cholesterol are carotenoid pigments and vitamin C, as an antioxidant, and hydrocolloid substances as a primary component (Julyasih *et al,* 2010 and Hernawati *et al,* 2013). Mean while, its carrageenan content has high potential in lowering cholesterol by inhibiting the absorption of cholesterol in the intestine (Subroto, 2011).

Taurine is an amino acid-2-ethanesulfonic which is a derivative of an amino acid found in many human organs, mammals, and marine animals (Huxtable, 1992). Taurine is an important component of bile salts and play a role in the absorption of fats and fat-soluble vitamins (Okuzumi and Fuji, 2000). Taurine is known to control cholesterol by increasing lipoprotein synthesis in the liver. Increased lipoprotein is proportional to the increase in HDL. HDL is needed to lowering the blood cholesterol or LDL in circulation, therefore it becomes an indicator of disease risk reduction hypercholesterolemia (Shim *et al,* 2009).

Therefore, this study was conducted to prove that the macroalgae (*Sargassum* sp., *Gracillaria* sp.) and taurine can lower total cholesterol levels of hypercholesterolemia male mice *(Mus musculus* L.) and to determine which from the three resources is able to lowering total cholesterol levels most in male mice.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Molecular Biology in Biology Department, Faculty of Mathematics and Natural Science, University of Lampung from February to March 2016.

Experimental Animals and Treatments

Forty 40 male mice with average weight of 20-40 grams were obtained from the Institute of Veterinary Lampung. Mice are devided into 8 treatment groups (C-, C+, CC, T1, T2, T3, T4, and T5) and each group contained 5 animals. All mice was acclimated for 7 days under laboratory conditions and fed with standard diet and drinking water *ad libitum*. The treatment consists of the group C- fed with standard until the 28th day, the group C+ were given a diet of hypercholesterolemia in the form of a suspension of brain cow 1 ml/day until the 28th day, the group CC, T1, T2, T3, T4 and T5 fed a diet hypercholesterolemia for 14 days, then on the 15th day CC group were fed with standard diet, group T1 were fed with standard diet and 15,6 mg/g BW of taurine, T2 group were fed with standard diet and 32 mg/g BW of *Sargassum* sp., T3 group were fed with standard diet and given a mixture of *Sargassum* sp. 32 mg/g BW and taurine 15,6 mg /g BW, the T4 group were fed with standard diet and 32 mg /g BW *Gracillaria* sp., and the T5 group were fed with standard diet and given a mixture of *Gracillaria* sp. 32 mg/g BW and turine 15,6 mg/g BW until the 28th day. The hypercholesterol diet were made by the method of Pratama and Probosari (2012), the dose of taurine solution were estimated by the method of Maysa (2016), and the *Sargassum* sp. and *Gracillaria* sp. solution were made by the method of Edriyansyah (2013).

Model and Data Analysis

After an overnight (8–12 h) fasted, during which only water was permitted, blood was collected from the tail. Some drop of blood was used to determine the total cholesterol levels using cholesterol test strips by *Nesco*. The hypercholesterolemia is indicated by more than 100 mg/dl cholesterol levels. Blood cholesterol levels and body weight were determined only at 0, 14^{th}, and 28^{th} day observation. Data were analyzed with ANOVA and followed by LSD test at α 5%.

RESULTS AND DISCUSSION

Average Body Weight of Mice *(Mus musculus* L.) During the Period of Study

Observations on the body weight of male mice showed some fluctuation as the effects of hypercholesterolemia and hypocholesterol diet, Figure 1.

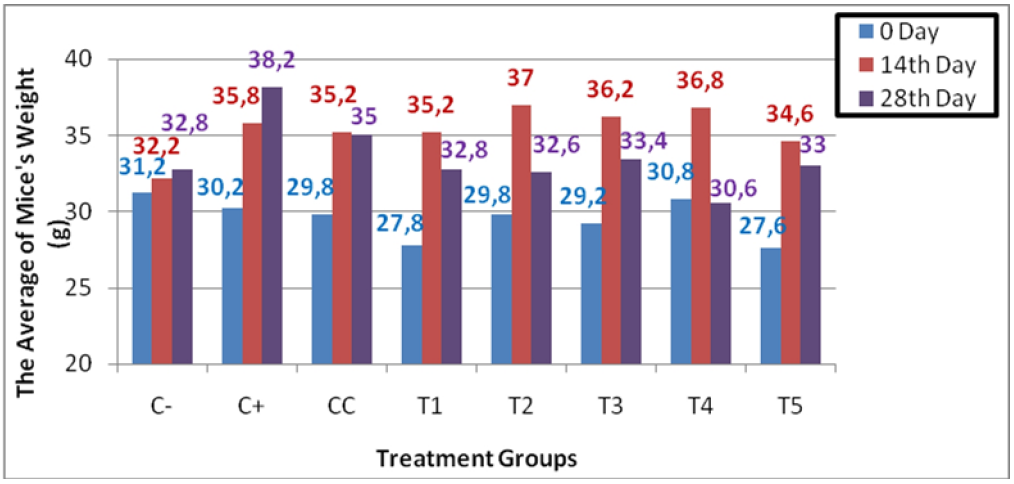

Figure 1. The mean average of mice body weight

Description: C-: negative control (standard feed for 28 days)
C+: positive control (cow brain suspension for 28 days)
CC: comparator control (14 days cow brain - 14 days standard feed)
T1: treatment 1 (14 days cow brain - 14 days taurine)
T2: treatment 2 (14 days cow brain - 14 days *Sargassum* sp.)
T3: treatment 3(14 days cow brain - 14 days taurine + *Sargassum* sp.)
T4: treatment 4 (14 days cow brain - 14 days *Gracillaria* sp.)
T5: treatment 5 (14 days cow brain - 14 days taurine + *Gracillaria* sp.)

The mean body weight of mice of all treatment groups increased up to 14 day observation, presumably all treatment groups experienced hypercholesterolemia except the C- group. On the 28th day, the group C+ continues to experience increased in body weight due to the hypercholesterolmic diet given up to 28 days, while group CC slightly decreased since the 14th day after hypercholesterolmic diet was replaced with a standard feed and distilled water until the 28th day. While in T4 group showed the highest weight loss up to 6,2 g in 28 days.

Total Cholesterol Levels in Mice *(Mus musculus* L.) After Induction to Hypercholesterolemic Diet

Total cholesterol levels of mice after induction to hypercholesterolemia diet for 14 days in each treatment group can be seen at Figure 2.

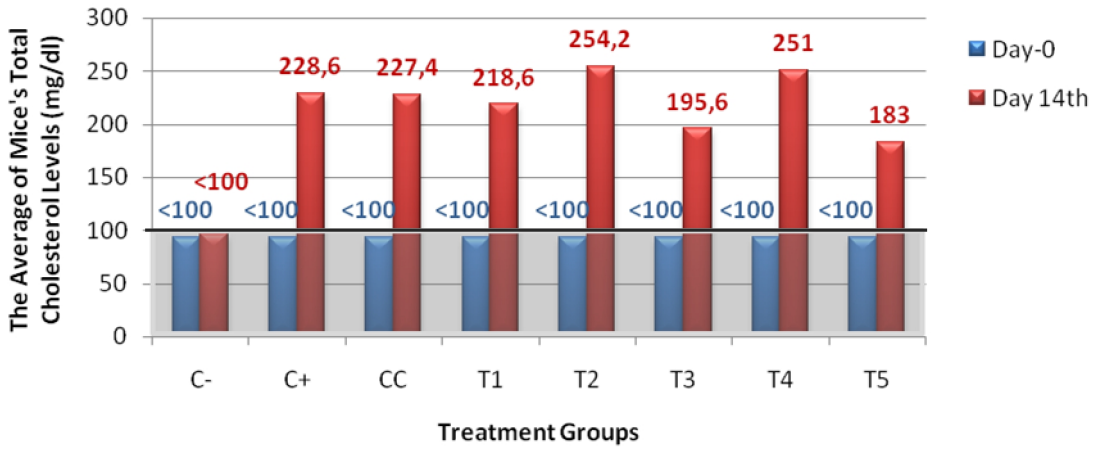

Figure 2. Average total cholesterol levels in mice after hypercholesterolemia treatment.

On the 14th day, all treatment groups showed significanty different compared to group C- which was only given standard feed (p <0,05), in which the increase of cholesterol levels was up to 50% - 100% (p <0,05).

Total Cholesterol Levels After Giving *Sargassum* sp., *Gracillaria* sp. and Taurine

The mean total cholesterol levels of mice on 28th day of each treatment group given *Sargassum* sp., *Gracillaria* sp. and taurine as well as the combination among three resources was able to significantly lowering total cholesterol levels of male mice (p <0,05) (Table 1).

Table 1. Average total cholesterol levels of mice (*Mus musculus* L.) after hypocholesterol treatment (at 28th day)

Groups	Mean ± Std. deviation	N
C-	100.40 ± 3.647^{e}	5
C+	296.40 ± 36.046^{a}	5
CC	223.40 ± 21.732^{b}	5
T1	206.40 ± 76.647^{bc}	5
T2	170.20 ± 28.199^{cd}	5
T3	170.60 ± 22.667^{cd}	5
T4	157.2 ± 51.276^{cd}	5
T5	152.4 ± 35.739^{d}	5

* Means followed by different superscript letters indicate significant differences based on test LSD 5%.

On the 28th day, blood cholesterol levels in mice C+ group continuously increased from day 0 and significantly different compared to the other treatment groups (p<0,05). While the C+ group had the highest average total cholesterol levels among others, 296,4 mg/dL.

The average total cholesterol levels of T2, T3, T4 and T5 groups showed significantly lower than C+ and CC groups, suggesting a decrease in cholesterol levels. Similarly, the T1 group decreased the mean total cholesterol levels significantly than C+ up to 30,4% (p<0,05) but not significantly different compared to CC that was equal to 7,6% (p> 0,05).

DISCUSSION

Fluctuations in the number of average weight and total cholesterol levels in the treatment groups demonstrated that administration of diets containing *Sargassum* sp., *Gracillaria* sp. and taurine could lead to weight loss and decrease in cholesterol levels of mice, after hypercholesterolemia diet was applied in this study. Hyperclolesterolmia diet contained of 2 grams of cholesterol and 2,9 grams of saturated fat (Pratama and Probosari, 2012), in which given 1 ml suspension of this diet induced aproximately 10 mg of cholesterol in experimental mice per day.

Foods high in cholesterol and saturated fats can increase levels of free fatty acids and triglycerides which can accumulate into body fat (Julyasih, 2012), therefore weight gain to the mice in this study was observed. Besides increasing body weight, triglycerides through a series of processes can be transformed into LDL in the circulation. LDL is a lipoprotein that is rich in cholesterol circulating in the blood vessels (Ganong, 2005). Jae (2008) also stated that foods high in cholesterol and saturated fat lead to a decrease in the transcription of the LDL receptor gene that resulting in decrease in LDL receptors synthesis. This caused increase in cholesterol levels of the blood.

The results showed that water can be unsignificantly lowering cholesterol and body weight of mice. It can be seen from the comparison control group which the mean decrease in the 28th day after the induction suspension cow's brain stopped on day 14 and replaced with a standard feed and drinking water *ad libitum* (Figure 1 and Table 1).

In the treatment of hypocholesterol groups the most highest in losing weight and total cholesterol levels in hypercholesterolemia mice was in the group fed with *Gracillaria* sp. 32 mg/gmBW and *Sargassum* sp. 32 mg/gmBW. The decline was presumably caused by the

high fibre content and the ability of carrageenan from *Gracillaria* sp. and alginate from *Sargassum* sp. which inhibited intestinal absorption of fat and cholesterol.

Carrageenan is one of the main products produced by seaweeds, especially in red algae (Rhodophyta). *Gracillaria* sp. is kind of *karaginofit* group because of its high product and as primary product in carrageenan such a polysaccharide (Hernawati *et al,* 2013). Carrageenan is a polysaccharide compounds and the water-soluble dietary fibre *(soluble dietary fibre)* (Carvalho *et al.,* 2009). The ability of carrageenan to imitate the texture and sensory quality of fat may reduce the total amount of fat in the diet and its function as a fat emulsifier better than gelatine contained *Gracillaria* (Panlasigui *et al.,* 2003).

Alginate is a good source of dietary fibre for health and largest content contained in brown algae, especially species of *Sargassum* sp. (Kadi, 2005). According to Wolever *et al.* (1997) hydrocolloid alginate compound from brown algae and high fibre food had proven to lower cholesterol levels in blood by binding to bile acids in the small intestine that causes increased an excretion of bile acids and bile acid synthesis. Increased excretion of bile acids makes an efforts to manufacture bile acids back to be lost, so that the heart will attract cholesterol circulating in the blood, eventually the blood cholesterol levels will decrease (Muchtadi *et al.,* 1993). Other the hand, allegedly the content of other active compounds such as flavonoids, saponins, alkaloids and tannins have a role in inhibiting pancreatic lipase activity which hydrolyze fat caused obesity (Rahardjo *et al.,* 2005).

Taurine is an amino acid derivative is known to reduce blood cholesterol (Kadam and Prabhasankar, 2010). Taurine is thought to lower cholesterol levels by increasing the activity of the enzyme *cholesterol 7-α-monooxygenase,* an enzyme that can stimulate the production of bile acids (Murakami *et al.,* 2006). Bile acids would bind with taurine to form bile salts and the remainder will come out with fecal excretion. The binding of bile acids and taurine as well as the continuous excretion resulting in heart will continue to take blood cholesterol for metabolism and secretion into the gall bladder, resulting in a reduction of blood cholesterol levels (Brownlee *et al.,* 2006). Then 5% taurine diet indicated can increase fecal excretion of bile acids and sterols for lowering cholesterol absorption in the small intestine (Sugiyama *et al,* 1989). While the excretion of bile acids increased, it is an attempt to reproduce the lost bile acids, the liver will pull the cholesterol circulating in the blood, so the blood cholesterol levels will decrease.

From this research we can conclude that as giving macroalgae (*Sargassum* sp. and *gracillaria* sp.) and taurine, as well as a mixture of macroalgae and taurine on diets can lower total cholesterol levels in hypercholesterolemia male mice *(Mus musculus* L.).

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THE EFFECT OF METAL IONS FE AND ZN EXPOSED TO MAGNETIC FIELD 0.2 MT ON THE PRODUCTION OF PROTEASE IN *Bacillus* Sp.

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ABSTRACT

One of the environmental factors, ie, the magnetic field may affect the microbes to produce enzymes. The ion Fe is ferromagnetic compound, used as inductor on the growth and activity of *Bacillus* sp. in producing the enzyme protease, whereas Zn which belong to the class of transition metal is diamagnetic can act as protease inhibitor. The purpose of this study was to determine the effect of metal ions Fe and Zn were exposed to a magnetic field of 0.2 mT to the production of proteases in *Bacillus* sp. The results showed that the culture medium containing metal ions Fe (a 0.01% w/v) were exposed by the magnetic field has a value of proteolytic index of 3.36 with 10 hour long incubation, and proteolysis index of 2.74 with 18 hour incubation time. Culture medium containing metal ions Fe (a 0.01% w/v) which are not being exposed to the magnetic field has a value of proteolytic index of 1.79 with 10-hour long incubation, and proteolysis index of 2.12 with 18 hour incubation time. While the culture medium containing metal ions Zn (0.005% w/v) either exposed or not exposed to the magnetic field causes the activity of *Bacillus* sp. die.

Key words: *Bacillus* sp., magnetic field, proteolytic index, and proteolysis index

INTRODUCTION

Modern biotechnology typically utilize the resulting microbial metabolites such as protease enzyme has been widely used in various fields of health, industry and livestock (Said and Likadja , 2012). Proteases are widely used in several industrial applications such as detergent additive substances, pharmaceutical industry, leather tanning, food industry, protein hydrolysates and sewage treatment plant (Purwadaria *et al.,* 2000). Protease is an important enzyme that is widely used in industrial applications and a 60% of total sales in the world's enzymes (Rao *et al.,* 1998). Source protease enzyme can be derived from animals, plants and microorganisms. However, in both economic protease production of animals and plants require resources and a big expense.

Microorganisms have a role and its own advantages in producing the protease enzyme because it can produce the enzyme in large-scale, short time, and relatively low cost (Kosim *et al.,* 2010). Various types of bacteria and fungi are reported capable of producing protease and some of them have been used on an industrial scale. One is bacteria of the genus Bacillus are constantly being developed to produce a protease enzyme (Sudaryati *et al.,* 2011).

Efforts should be made to optimize the activity of *Bacillus* sp. in producing the protease enzyme is to treat them as the magnetic field on the Fe and Zn were used as inductors growth of *Bacillus* sp. Research on the role of magnetic fields in microbiology today began much done. The magnetic field has been reported to affect the activity of the enzyme peroxidase, catalase and superoxide dismutase (Lie*.,* 2015). Fe as a ferromagnetic material having an atomic resultant magnetic field is great. In the Fe many unpaired electrons that will cause the magnetic field at each of the electron spins (Dilawar *et al.,* 2008). While Zn is a transition metal ion that can decrease the value of coercivity (magnetic intensity) of a material so that the material will be soft magnetic (Sholihah *et al.,* 2012).

Therefore, in this study will be seen how the effect of exposure to 0.2 mT magnetic fields on the metal ion Fe and Zn to the induced power in the activity of *Bacillus* sp. in generating enzyme protease and know aktiitas protease produced by *Bacillus* sp..

MATERIALS AND METHODS

Materials needed is for bacteriologi, yeast extract, NaCl, KH_2PO_4, $MgSO_4$, $(NH_4)_2SO_4$, $FeCl_3$, $ZnCl_2$, substrate casein, TCA, a buffer solution, tyrosine, Folin reagent. While the equipment used is a set of glassware, petri dish, autoclave, selonoid 0.2 mT, vortex, UV spectrophotometer, oven, incubator, Laminar Air Flow (LAF), scales, centrifuge.

Mendels Modification Culture of *Bacillus* sp.

Media Mendels modification consists of materials: a) NaCl 0.1 g, yeast extract 0.175 g, KH2PO4 0.1225 g, MgSO4 0.0175 g, (NH4) 2SO4 0.0875 g, so bacteriologi 0.75 g, all the ingredients are dissolved in 35 ml of distilled water, b) total 12:25 g milk dissolved in 5 ml of distilled water, c) a 0.01% FeCl3 and 0.005% respectively ZnCl2 were dissolved in 5 ml of distilled water. 0.2 mT magnetic field is exposed to FeCl3 and ZnCl2 solution for 10 minutes. Furthermore, all the ingredients a, b and c poured in Erlenmeyer's first homogenized and then used.

Isolation and Selection of Protease Producing Bacteria

Bacillus sp. isolated from chicken intestine by cutting 1 cm intestine chicken is then inserted into a modified liquid medium Mendels then the culture was heated at 80ºC for 15 minutes and incubated at a temperature of 37ºC for 2 x 24 hours. Selection of protease-producing bacteria is done by: a single ose bacterial suspension was inoculated on a solid media Mendels modified with casein substrate and then incubated at a temperature of 37ºC for 24 hours. Positive results were marked by a clear zone around the growth of bacterial colonies. Conversely, a negative result is characterized by the absence of a clear zone around the growth of bacterial colonies.

Determination of protease activity is based on the value of proteolytic index (IP)

Proteolytic Index (IP) is a measure that indicates the ratio between the diameter of the clear zone of the colony diameter (Durham *et al.,* 1987). IP value ≥3 isolates showed that the isolates had great potential as a source of protease and maximum (Said and Likadja , 2012). Proteolytic index can be calculated using the formula:

$$IP = \frac{B}{A}$$

Get:
IP: Proteolytic Index
A: The diameter of the colony
B: The diameter of the clear zone (Sumardi *et al.,* 2010).

Isolates of *Bacillus* sp. Mendels grown on solid media were modified with as many as six different treatments. The treatment given is as follows:

Treatment 1 (M_0L_0). Treatment M_0L_0 a control treatment where Mendels modified solid media were not given exposure to magnetic fields and are not given the inductor.

Treatment 2 (M_1L_0). M_1L_0 behavior is treated using a modified solid media Mendels and 0.2 mT magnetic field exposed for 10 minutes but without any inductors.

Treatment 3 (M_0L_1). M_0L_1 treatment is treatment using dense media Mendels modified and given inductor $FeCl_3$ with a 0.01% concentration of media. Good and inductors are not being exposed to a magnetic field.

Treatment 4 (M_1L_1). M_1L_1 treatment is treatment using dense media Mendels modified and given inductor $FeCl_3$ with a 0.01% concentration. $FeCl_3$ was exposed to 0.2 mT magnetic field for 10 minutes before use.

Treatment 5 (M_0L_2). M_0L_2 treatment is treatment using dense media Mendels modified and given inductor $ZnCl_2$ with a concentration of 0.005%. Neither the media nor the inductors are not being exposed to a magnetic field.

Treatment 6 (M_1L_2). M_1L_2 treatment is treatment using dense media Mendels modified and given inductor $ZnCl_2$ with a concentration of 0.005%. $ZnCl_2$ was exposed to 0.2 mT magnetic field for 10 minutes before use.

RESULT AND DISCUSSION

Bacillus sp. produced of Protease

Proteolytic activity of *Bacillus* sp. on solid media Mendels modified seen from the formation of a clear zone around bacterial colonies growing. Selection media used in this study is a modified media Mendels and milk as a substrate. Milk contains casein, a suitable medium for the growth of proteolytic bacteria because it is rich in nutrients (Zahidah and Maya, 2013). With the conclusion of the enzyme protease by *Bacillus* sp. This will hydrolysed casein into smaller peptides and amino acids marked by the formation of a clear zone around the colony. The more widespread that form a clear zone indicates that the bacteria have a high ability to change the substrate contained in a growth medium (Vijayaraghavan *et al.,* 2013).

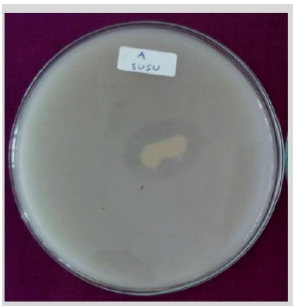

Figure 1. The clear zone formed around colonies of bacteria. A: Clear Zone, B: Colonies of *Bacillus* sp.

Proteolytic Index (IP)

In addition to visits from the presence or absence of a clear zone, the ability of *Bacillus* sp. proteolysis can be seen from the proteolytic index (IP). The results of calculation of proteolytic index (IP) *Bacillus* sp. of six treatments are presented in the following table.

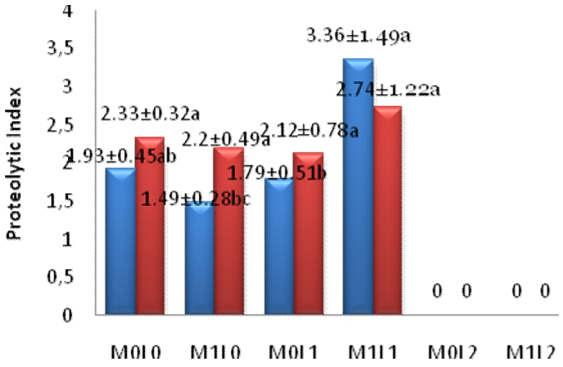

Figure 3. Value Index proteolytic (IP) Bacillus sp. on solid media without treatment magnetic field (M0), the treated magnetic field 0.2 mT for 10 minutes (M1), a solid medium does not contain metal ions Fe and Zn (L_0), media containing metal ions Fe (L_1), media containing ion Zn metal (L_2).

Figures followed by different letters show significant difference in each treatment.

The result of the calculation of the index value proteolytic (IP) of the six treatments showed proteolytic index values vary both in the incubation time of 10 hours or 18 hours. This is because the difference in treatment given. Isolates in treatment M_1L_1 ie bacteria grown on solid media Mendels modified and given inductor $FeCl_3$ with konsentrasi a 0.01% which has been exposed by the magnetic field 0.2 mT for 10 minutes has an index value of proteolytic highest good in the incubation time of 10 hours and 18 hours are consecutive 3.36 and 2.74. This is presumably due to the addition of $FeCl_3$ that has been exposed by a magnetic field so that power inductor will be a positive influence on the proteolytic activity of *Bacillus* sp..

When $FeCl_3$ that has been exposed by the magnetic field was added to the bacterial growth media stored on the magnetic properties will affect the growth of bacteria that grow on these media. As reported by Sudarti *et al*. (2014) that the magnetic field can directly influence the metabolic activity of cells one to produce the enzyme. Farisna research results and Enny (2015) explains that *Bacillus* sp. able to grow on solid media containing Fe as Fe is needed in the metabolism of bacterial cells even though in small amounts.

In the treatment M_0L_2 and M_1L_2 have proteolytic index value (IP) 0 by the time of incubation of 10 hours or 18 hours. This is because the treatment M_0L_2 and M_1L_2 no bacterial growth and a clear zone formed by the addition of $ZnCl_2$ with a concentration of 0.005%. The metal ion contained in $ZnCl_2$ salt can damage the cell membrane permeability of bacteria that cause bacterial growth be inhibited so as to affect the production of the enzyme (Trianie, 2014).

In the treatment M_1L_2, $ZnCl_2$ used have been given exposure to 0.2 mT magnetic field for 10 minutes but no effect on the activity of *Bacillus* sp. because Zn is a transition metal ion is diamagnetic which could lower the value of coercivity (magnetic intensity) of a material so that the material will be soft magnetic (Sholihah *et al.,* 2012). In diamagnetic materials virtually all unpaired electron spin, as a result of this material does not draw a line style (Halliday and Resnick, 1989).

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GENETIC VARIABILITY AND HERITABILITY OF VEGETATIVE AND GENERATIVE TRAITS OF DIFFERENT SORGHUM GENOTYPES

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ABSTRACT

The development of sorghum varieties have been conducted with several purposes such as to obtain grain sorghum, sweet sorghum, forage sorghum, and biomass sorghum. Recently, sorghum breeders try to develop sorghum type for bioenergy. Unfortunately, there is a little information on genetic variability especially that of biomass sorghum. The objectives of this study were to evaluate the genetic components of 20 different sorghum genotypes, to estimate the broad sense of heritability value, and to assess the economic yield for biofuel and feed. There were 20 sorghum genotypes as GH-1, GH-2, GH-3, GH-6, GH-7, GH 9, GH 10, GH 11, GHP-1, GHP-3, GHP-11, P/I WHP, P/F 5-193-C, PF 10-90-A, P/I 150-21-A CYMMIT, Mandau, Numbu, Pahat, Talaga Bodas, UPCA. Treatments were arranged in randomized block design with 3 reps. Variables observed in this study were root dry weight, leaf number, leaf dry weight, stem dry weight, inflorescence length, and yield components as seed size and seed weight. Data were analysed by using analysis of variance and broad sense heritability. Analysis of variance showed that genotypes were significantly different based on the variables observed. Plant height, panicle length, and seed size showed high broad sense heritability i.e. 90.2, 92.6, and 92.3% respectively. On the other hand, stem diameter, seed number and seed weight have low broad sense heritability i.e. 52.3, 54.5, and 55.7% respectively.

Keywords: biomass, broad sense heritability, genetic variability, sorghum

INTRODUCTION

The development of breeding program in sorghum (*Sorghum bicolor* (L) Moench) have been conducted with aims of obtaining different type of sorghum such as grain sorghum, sweet sorghum, forage sorghum, and biomass sorghum. It is well documented that sorghum has a genome size of 736 Mb and is diploid. Its relatively small genome makes it a suitable model for other related crops with much larger genomes or polyploidy, such as sugar cane.

Sorghum has 25 species with the chromosome number of 2n=2x=20 (Wang *et.al*. 2015). Recently, breeders tend to focus on developing sorghum for feed and biofuel. They have also been developing sorghum variety not only for high sugar content but also for cellulose content. The information regarding the social economic (Amosson, et.al. 2013; Saptoadi. 2015), agronomic (Bakheit, 1990; Fujii, et.al. 2014; Hoffmann and Rooney. 2014; Pabendon *et.al*. 2012; Purnomohadi. 2006; Rocateli *et.al*. 2012; Hadi *et.al*. 2016; Setiawan *et.al*. 2016), physiological (Barbanti *et.al*. 2015; Beheshti and Fard. 2010) and genetic (Setyowati *et.al*. 2005; DeLacya *et.al*. 2010; Singh *et.al*. 2012) characters of sorghum has already been published. DeLacya *et.al*. (2010) reported that the trait for high grain yield tended to associate with early flowering, whereas high stover yield would associate with plant height. Kong *et.al*. (2014) showed that based on identified QTL of sorghum population, the genetic determinants of branching might contribute to components of vegetative branching in sorghum. It seems that trait of stem characters is concomitant with stem improvement for biofuel and feed. However, the published papers on genetic characters of selected sorghum genotypes for biomass production are still rare. The objectives of this study were to evaluate the genetic components of 20 different sorghum genotypes, to estimate the broad sense of heritability value, and to assess the economic yield for biofuel and feed. What it means by the economic yield this paper is seed number, seed size, and seed weight, harvest index and biomass index.

MATERIALS AND METHODS

This study was conducted from April to July 2015 in dry land of South Lampung with planting materials of 20 sorghum genotypes. Treatments were arranged in randomized block design with 3 reps. Three seeds per hole were planted with space of 80 cm x 20 cm. Fertilizers as urea, TSP and KCl were used in this study with the dosage of 150 kg/ha, 100 kg/ha, and 150 kg/ha, respectively. Fertilizers of urea and KCl were applied twice in 30 day after planting (DAP) and 60 DAP for half dosage, while TSP was applied once in 30 DAP.

Variables observed in this study were root dry weight (RDW), leaf dry weight (LDW), stem dry weight (SDW), panicle length (PL), panicle dry weight (PDW), and yield components as seed size (SS), seed number (SN), seed weight per panicle (SW), leaf number (LN), plant height (PH), stem diameter (SD). Photoassymilate allocation was determined by harvest index (HI) and biomass index (BI). Seed size was calculated from 100 seeds (g). HI

was based on ratio between SW and total of RDW, LDW, SDW, PDW and SW. Biomass index (BI) was calculated as SDW divided by RDW, LDW, SDW, PDW and SW. The grouping for sorghum biomass production type was based on intensity of the value of BI that is ≥ 50. Data was analysed by using SAS 9.0 including analysis of variance.

Broad sense heritability (H^2_{bs}) was calculated and modified from Stansfield (1991) as the followings: (H^2_{bs}) = (σ^2g/σ^2f) x 100% where 40% < H^2_{bs} < 55% is small; 55% < H^2_{bs} < 70% is medium and H^2_{bs} > 70% is high.

σ^2g (variance of genetics) = {(MSG – MSE)/n} and then

σ^2f (variance of phenotype) = $\sigma^2g + \sigma^2e$

σ^2e is the same as MSE

Standard deviation of genetic variance is followed:

$\sigma\,\sigma^2g = \sqrt{(2/r^2)}\{(MSG/dfg) + 2) + (MSE/dfe) + 2)\}$ where dfg is degree freedom of genetic and dfe is degree freedom of error.

The Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were determined to indicate the variation performance in the field by formula as followed:
(GCV) = $\sqrt{}$ (σ^2g/X) x 100% and (PCV) = $\sqrt{}$ (σ^2f/X) x 100% where X = general means
CV < 30% is narrow; 30 < CV < 40% is medium and CV > 40% is wide

RESULTS AND DISCUSSION

Based on vegetative and generative variables of 20 sorghum genotypes used in this study, the genetic variance was considered to be high. According to Ng'Uni et.al (2011) by using simple sequence repeat (SSR), genetic variation was revealed according to the sites of sorghum collection. Additionally, the range values of H^2_{bs} were 52-93% (Table 1). Most of the traits observed in this study showed high H^2_{bs} except for SD, SN, and SW. Three traits of SD, SN, and SW had the values of H^2_{bs} as 52.3, 54.5, and 55.7%, respectively. It implies that most of the characters that showed high H^2_{bs} were under genetic control. The variable of SD showed low value of H^2_{bs} as 52.3% compared to plant height that was 90.2%, suggesting that trait for plant height of sorghum would be more heritable than that for SD. This result was similar to the DeLacy *et.al*.'s study in 2010 who reported that the heavier stover production was closely related to taller sorghum type. Moreover, this result was also associated with the previous result (Setiawan *et.al*. 2016) that plant height was significantly correlated with SDM

of unselected sorghum genotypes based on the best performance of biomass production group as r=0.61*. However, this correlation would not be significantly different for sorghum genotypes which were selected for the best performance of biomass production group. This condition was probably caused by highly homogenous vegetative characters selected for the best performance of biomass. This inferred that stem characters as length and internode seem suitable trait for selection for improvement of biomass sorghum. On the contrary, SD does not appear to be a proper trait for selection for biomass sorghum. Besides, SD showed low H^2_{bs} (52.3%) and seemed to be influenced by environmental factors.

Three traits of SD, SN, and SW having low H^2_{bs} are under environmental factors. It means that such traits would be influenced by environmental factors. Three generative traits (PH, PL and SS) showed high H^2_{bs} i.e. 90.2, 92.6 and 92.3%, respectively, suggesting that these traits were mostly controlled by genetic factors. Two sorghum genotypes of GH-2 and GH-6 revealed high BI (0.51). This infers that these sorghum genotypes have heavier biomass compared to other sorghum genotypes in this study. Accordingly, these genotypes could be used as a donor to improve biomass traits especially plant height. Two traits of PL and SS seem directly contribute to seed number and seed weight that lead to influence HI. Seed number and seed weight were two traits that as individual variable do not directly affect the HI because many factor affected HI as SDW, LDW, PDW, and RDW.

Among variables observed in this study, SN had high SDGV and this would directly affect very high CV of both phenotype and genotype (Table 2). It seems that such condition was influenced by high range of SN that was 1200-3086 (Table 3). The very high difference between GCV and PCV was revealed in SN as 46.08 (141.43-95.35). The cause of this is still not very clear yet. However, high SDGV of SN might contribute to high differences between GCV and PCV as 82.01. It means that 20 sorghum genotypes tested in this study produced high variation in SN. The result of this study was not the same as that of Jain and Patel (2013) who reported that stem girth showed highly different between GCV and PCV.

Two sorghum genotypes that produced high biomass were GH-2 and GH-6 as 0.51 (Table 3). Both of these sorghum genotypes produced low yield of SW that was less than 70 g per panicle. The lowest biomass production was revealed by GHP-3 as 0.05 yet this sorghum genotype is able to produce high yield (SW= 97.8 g per panicle). Based on this, GH-2 and GH-6 could be used for biomass production yet GHP-3 would be suitable for grain sorghum.

Genetic variation of 20 sorghum genotypes was high. Three phenotypic traits showing high H^2_{bs} were height (90.2%), panicle length (92.6%), and seed size (92.3%). Stem diameter, seed number, and seed weight tended to have low broad sense heritability i.e. 52.3, 54.5, and 55.7%, respectively. The value of standard deviation of genetic variance for seed number was very high (82.01) and this would lead to associate with high CV of phenotype and genotype. This study would recommend that GH-2 and GH-6 have good economic values because these sorghum genotypes could be used for biomass production for both biofuel and feeds. Therefore, both of GH-2 and GH-6 are sorghum genotypes that could be used as parents for biomass trait improvement in a breeding program.

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Table 1. Mean square of genotype (MSG), mean square of error (MSE), variance of genetics, and heritability of broad sense (H^2_{bs}) of 20 sorghum genotypes.

Variables	MSE	MSG	Variance of Genetics	H^2_{bs} (%)
RDW	71.295	11.162	20.04	64.23
SDW	3911.450	185.917	1241.84	86.98
LDW	44.925	7.816	12.37	61.28
Plant Height	12767.441	445.916	4107.17	90.21
LN	3.683	0.253	1.14	81.91
SD	0.080	0.019	0.02	52.32
PDW	15.081	1.227	4.62	79.01
PL	47.444	1.226	15.41	92.63
SS	0.960	0.026	0.31	92.30
SN	798190.710	173533.720	208219.00	54.54
SW	908.735	190.167	239.52	55.74
HI	0.046	0.003	0.01	83.74

RDW= root dry weight, SDW= stem dry weight, LDW= leaf dry weight, LN= leaf number, SD= stem diameter, PDW= panicle dry weight, PL= panicle length, SS= seed size, SW= seed weight, HI= harvest index

Table 2. Standard deviation of genetics variance (SDGV), general means, coefficient of variation (GCV) for genetics, and coefficient of variation (PCV) for phenotype of 20 sorghum genotypes.

Variables	SDGV	Means	CV of Phenotype	CV of Genotype
RDW	2.264	11.932	16.17	9.67
SDW	7.392	66.644	46.29	16.70
LDW	2.175	23.140	9.34	5.81
Height	12.848	209.613	46.61	14.59
LN	2.012	12.173	3.39	1.44
SD	2.000	1.804	1.47	1.02
PDW	2.052	7.447	8.86	4.06
PL	2.142	22.161	8.66	2.35
SS	2.003	3.791	2.98	0.83
SN	117.925	1908.567	141.43	95.35
SW	4.431	71.143	24.58	16.35
HI	2.000	0.432	2.00	0.80

Tabel 3. The means of generative variables as seed size (SS), seed number (SN), seed weight (SW), harvest index (HI), and biomass index (BI) form 20 sorghum genotypes

Genotype	SS (g)	SN	SW (g)	IP	BI
GH-10	3,67fg	2242,7b-e	100,1ab	0,34d-f	0,45abc
GH-7	4,10cd	1821,7c-g	74,8b-f	0,31efg	0,47ab
P/F5-193	3,33bc	3086,7a	107,5a	0,40cd	0,39b-e
GH-2	4,30bc	1355,2fg	56,9d-g	0,27g	0,51a
GH-1	4,07cde	1471,0efg	58,2d-g	0,30fg	0,48ab
Mandau	3,90def	1690,5d-g	72,4c-g	0,34d-g	0,44a-d
GH-6	3,80ef	2827,7ab	46,5g	0,26g	0,51a
P/IWHP	4,50ab	1207,2g	51,0fg	0,28g	0,48ab
GH-3	4,50ab	1620,3d-g	70,6d-g	0,37c-f	0,38b-e
GH-9	4,17cd	2053,2b-f	81,1b-e	0,44c	0,34cde
Talaga Bodas	4,60a	1772,3d-g	78,8b-e	0,43cd	0,47cde
Numbu	4,57ab	1560,3d-g	72,2c-g	0,40cde	0,34de
GH-11	2,80j	2109,5b-f	61,2d-g	0,37c-f	0,37b-e
P/F10-90	3,10i	1722,8d-g	54,0efg	0,39c-f	0,36cde
UPCA	4,05cde	1523,0efg	63,2d-g	0,42cd	0,30ef
Pahat	3,41gh	2371,7a-d	83,4a-d	0,53b	0,22fg
GHP-11	3,08i	2075,0b-f	80,4b-e	0,60c-f	0,17g
P/I150-2	3,08i	1207,0g	46,5g	0,54b	0,16g
GHP-1	3,32hi	1865,0c-g	66,1d-g	0,56b	0,13gh
GHP-3	3,47gh	2588,7abc	97,9abc	0,68a	0,05h

Number in the same column followed by the same letter indicated not significantly difference under Tukey's procedure in the level of 5%

RESILIENCE IN THE FACE OF CHANGING CLIMATE: THECASE OF INDIGENOUS *BAGOBO* COMMUNITIES, DAVAO, MINDANAO, PHILIPPINES

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Running Title: Climate Change Adaptation of Bagobo Communities

Keywords: climate changeadaptation, indigenous peoples, indigenous knowledge, Bagobo

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ABSTRACT

The Philippines, one of the most vulnerable countries to the adverse impacts of climate change, is a culturally diverse country with more than 14 million Indigenous Peoples (IPs). Their rich knowledge systems can be tapped to provide solution to many adaptation and mitigation measures to CC, hence a research to document and communicate the IK for CC adaptation of selected IPs. The *Bagobo-Klata* and *Bagobo-Tagabawa* of Davao have experienced warmer climates, longer dry periods, more intense pest outbreaks, erratic rainfall pattern, and increased frequency and intensity of typhoons in the recent years. Their adaptive capacities and practices in response to changing climate are to ensure stability of supply, accessibility and availability of food and income sources throughout the year. They practice biodiversity-based farming systems, conservation and use of diverse traditional crop varieties, plant typhoon- and drought-resilient crops, consume lesser-known underutilized indigenous food crops, and use local knowledge of stars and constellations and other indicators and warning systems in deciding what and when to plant and harvest specific crops. It is therefore important to come up with policies and programs to support the *Bagobo* and to make use of their knowledge system and increase their resilience to climate variability and change.

Keywords: climate changeadaptation, indigenous peoples, indigenous knowledge, Bagobo

INTRODUCTION

The Philippines is composed of 7,017 islands. Located in Southeast Asia (13 00 N, 122 00 E), it is bounded in the north by Taiwan, in the west by Vietnam, in the south by Indonesia and in the east by the Philippine Sea (Figure 1). Its position on the Pacific Ring of Fire and its tropical climate make the Philippines prone to earthquakes and typhoons but have also endowed the country with natural resources and made it one of the richest areas of biodiversity in the world.

Being an archipelagic country with high poverty incidence, the county is one of the most vulnerable countries to the adverse impacts of climate change.Climate change (CC) impacts like increasing temperature, sea level rise, saltwater intrusion, and increasing occurrence of climate variability and extremes like typhoons and earthquakes (Comiso et al, 2014)have already been experienced in the recent past. Reports are replete that climate change is the next challenge to all societies and that the need to prepare for this challenge is imperative. Otherwise, the goals of sustainable development will surely be undermined.

The Philippines is categorized broadly into three main geographical divisions: Luzon, Visayas, and Mindanao, with Manila as its capital city. Mindanao is a mountainous island in the southernmost part of the Philippines and is the second largest at 104,630 km^2. Normally, the region lies outside the tropical cyclone belt with frequency of only 1 typhoon in 60 years, based on the 1948-2010 data. However, in 2012, Davao Oriental was hit by Tropical Storm (TS) Pablo (International name: Bopha), the deadliest tropical cyclone that battered the area, then by TS Titang (International name: Kate), the super typhoon that hit the southern part of Davao in October 1970, or 46 years ago.Davao regon and areas of Central Mindanao are prone to flooding and earthquake hazards (PAG-ASA, undated).

The Philippines is a culturally diverse country with an estimated 14-17 million indigenous peoples (IPs) belonging to 110 distinct ethno-linguistic groups (Cariño, 2012). The Bagobo constitutes one of the largest groups among the indigenous peoples of southern Mindanao. They are composed of three (3) sub-groups, namely the Tagabawa, the Klata or Guiangan and the Ubo. Although they belong to one socio-linguistic group, theydiffer in some ways, such as dialects, dance steps, costumes and their color preferences, among others. The Bagobo are the predominant inhabitants of the vast areas extending from the west coast of Davao Gulf to Mt. Apo, the highest peak in the Philippines at 3,146 masl. To the Bagobo, Mt. Apo or Apo Sandawa is sacred grounds and is valued among the group's richest cultural

heritage. It is also the home of world-known Philippine eagle (*Pithecophaga jefferyi*) (Mangune, 2015).

In general, IPs had survived over millennia in the face of climatic uncertainties and unpredictable extreme weather events, evidence that they are in intimate relationship with their environment and have sound knowledge of their environment and how to cope with it. The bases of their rich local practices and adaptation measures are context-, culture-, and location-specific; their rich knowledge systems and practices can be tapped to provide solution to many adaptation and mitigation measures to climate change. This is the raison d'être for a recently concluded research project that aimed to document and communicate the indigenous knowledge (IK) for climate change adaptation (CCA) of selected IPs in the Philippines. Specifically, this paper aims to document and validate IK practices for CCA of two Bagobo indigenous communities of Davao City and Sta. Cruz, Davao del Sur.

METHODOLOGY

A government funded research project entitled "Documentation of Indigenous Knowledge for Climate Change Adaptation" was conducted from May 2011 to January 2016. The research covered five provinces and eight different indigenous peoples. The Bagobo-Klata and Bagobo-Tagabawa communities are two of the eight IP groups in this said research.

Formal linkage with National Commission on Indigenous Peoples (NCIP) Region XI and the Department of Social Science of the University of the Philippines (UP)Mindanao was established in March and December of 2011, respectively. NCIP identified and facilitated communication with the Bagobo-Tagabawa of Barangay[1] Tibolo, Sta Cruz while UP Mindanao identified and facilitated communication with the Bagobo-Klata of Barangay. Manuel Guianga,Tugbok District, Davao City.

Initial visit and discussions with the respective tribal councils of the identified indigenous communities, to ask permission to be included in the study, was made during the first week of July 2011 for the Bagobo-Tagabawa and one year later for the Bagobo-Klata. In coordination with the regional NCIP, a Memorandum of Agreement and Resolution allowing for the conduct of the research was obtained from each Bagobo community.

[1]village

The actual documentation of past/actual/existing indigenous practices for climate change adaptation was conducted in October 2011 and March 2012, for the Bagobo-Tagabawa and Bagobo-Klata, respectively. A guide questionnaire was developed and used for the documentation while photos and videos were taken during the process. Documentation was done through non-structured interviews of key informants, focused group discussions, and actual observation of the area/people.

Climate data for the past 31 years (1979-2009) was gathered from the National Center for Environmental Prediction (NCEP) Climate System Reanalysis (CFSR) (http://cfs.ncep.noaa.gov/cfsr). Secondary agricultural and demographic data were also gathered. Primary and secondary information gathered during the documentation were collated and summarized while a series of data validation, through presentation of the results to the concerned community, were conducted on site in April of 2014. Appropriate changes were made on the final documentation based on the validation process. Documentation and validation were both conducted in the local Cebuano dialect and were translated to English for the final report.

RESULTS AND DISCUSSION

Climate

The climate of Davao region is Type IV, based on the Corona climate classification system, which is characterized by rainfall evenly distributed throughout the year. The lowest amounts of precipitation were experienced during the period 1990-1999 while the 2000-2009 data is comparable with the baseline record in 1980-1989 except for a significant increase during May and a decrease during February (Figure 2). There was an observed increase in maximum temperature during the period 1990-1999 during the months of July to October. For minimum temperature, there was a significant increase during the period 2000-2009 for the months January to April and towards the end of the year on November and December.

The Bagobo-Klata Of Davao City

The Bagobo-Klata resides in BarangayKahusayan, Manuel Guianga, Tugbok District, Davao City (7°5'14"N 125°24'8"E)(Figure 3).Tugbok District has 18 villages with land area of 15,391 hectares or 6% of the total land area of Davao City. The Bagobo-Klata's ancestral domain is located at a high elevation and has cool climate conducive for farming.

The Bagobo-Klata or Guianganconsists of only 50 households with the young Datu

Danny Diarog as its tribal leader. Almost all the adults are working in an adjacent Sumifru banana plantation.In general, the Bagobo-Klata marry at an early age of 14-16. In the past, only the men work in the farm while the women stay at home and take care of the children and do household chores. At present, women help men in the farm work and are also engaged in sewing, washing clothes for a fee, and planting of vegetables. In terms of education, finishing high school is the only goal.

Rice and vegetables are the usual meal taken on a daily basis by the Bagobo-Klata. Water for drinking and household use is sourced from the nearby waterfalls. The forest in the area is still intact and where a lot of rattan (*Calameae*)grows abundantly.

Climate Change and Adaptation of the Bagobo-Klata

The observed climate changes in the area are unpredictable occurrence of rainfall and increased temperature. In the past, dry and wet months are predictable such that people then could prepare on time when to clear the land and plant their crops. An example of unpredictability is that March used to be a dry month, but now, it has become a rainy month. Sometimes it is hot half of the day then it rains on the other half. In 2011, the area experienced three full months without rain. There was no fog even in the mountains during that time and the sky was blue during daytime, indicating that temperature was quite high.

The climate became so unpredictable that the Bagobo-Klata farmers do not know when is the right time to plant their crops. Crop diversification,in terms of species and varieties planted, is one adaptation of the Bagobo-Klata to the changing climate. Aside from abaca (*Musa textilis*), which is their main crop, root crops[2] [sweet potato(*Ipomoea batatas)*, cassava[3] (*Manihot esculenta*), purple yam (*Dioscorea alata)* and taro (*Colocasia esculenta*)], cereals [rice[4] and corn[5]], vegetables [chayote (*Sechium edule*), pechay (*Brassica rapa*), bottle gourd (*Lagenaria siceraria*), tomato (*Solanum lycopersicum*), eggplant (*Solanum*

[2]*Katimpa, manabang* and *lapis* are the sweet potato varieties still presently being planted. *Katimpa* has big white tubers that can be harvested in one month's time while *manabang* and *lapis* have red and violettubers, respectively. These are planted mainly for home consumption.

[3]The white cassava is more delicious and demands a higher price while the yellow one is quite hard and is more affected by climate change. In the past, when the area was still forested, cassava growth was more vigorous and woody and would be left to grow taller than people.

[4]Lowland rice is planted during the dry season and not during the rainy season. This is because the community is located in a high elevation such that humidity is high enabling rice to survive.

[5]Two types of corn are grown, the white and the yellow. White corn or *tiniguib* and rice are produced for consumption purposes while the yellow glutinous corn is for the market.

lycopersicum), cabbage (*Brassica oleracea* var. capitata), chili (*Capsicum annuum*), pole sitao (*Vigna unguiculata* ssp. *sesquipedalis*) and other legumes, radish (*Raphanus sativus*) and cucumber (*Cucumis sativus*)], banana (*Musa* sp.), coconut (*Cocos nucifera*), coffee (*Coffea* sp.)and fruit trees [santol (*Sandoricum koetjape*), marang (*Artocarpus odoratissimus*), avocado (*Persea americana*) and guava (*Psidium guajava*)] are also planted.

Diversity is also observed in farm animalsfound in the area. These are goats, horses, carabao, chicken[6] and pigs thatare sources of food and income in times of crop failure.

Changing the type of crop planted and the use of chemical inputs are the adaptations made by these farmers in response to the unpredictable occurrence of rainfall. Some farmers reported that there are areas in their farms wherein the soil has become so degraded that even the use of chemical fertilizers does not ensure a good yield anymore. Another adaptation is to concentrate on their abaca production, the one crop that does not need any chemical inputs.

Bagobo-Klata farmers also observed that temperature now is hotter than it used to be. In the past, they can still work and walk around even at high noon because the heat was still tolerable and not painful upon contact to the skin. Now, the heat is so intense that they need to stay under the shade between 12:00 noon and 2:00 pm. This has resulted in adjustment in the time that they work in the farm, avoiding sun exposure during these times.

In general, the area did not experience any El Nino incident although there were times when their rivers dried up. In 1995, a strong typhoon hit the area. The Bagobo-Klata observed that the cool temperature of the high elevation areas is not as cool as it used to be. In terms of flooding, the worst flooding was experienced in mid-February of 2012.

Several landslides triggered by intermittent heavy rains, were observed at about three times monthly in the area, starting in 2008. In the past, two days of continuous rains or one month of intermittent rains would not result to landslides, even in sloping areas. Now, even a single day of light continuous rains could trigger a landslide. For the Bagobo-Klata, the sowing of seeds and planting of wildlings of native hardwood trees such as almasiga (*Agathis philippinensis*), karengag (*Cinnamomum* sp.), bansilay (*Cratoxylum* sp.), blising and sedar (*Dacrycarpus* sp.) are the only permanent solutions to landslides. After planting, the landslide areas are left to fallow and about ten years later, these will revert back to a forest. In addition,

[6]White chicken is used in rituals (*panubad-tubad*), as medicine, and as a sacrifice when asking permission to hunt in the forest. Horses are mainly used for transport of produce while carabaos are draft animals. Native pigs are cooked during pre-marriage arrangements (*pamalayi*) and other important events.

tiger grass *(Thysanolaena maxima)* is planted near the riverbanks to prevent soil erosion caused by floods. This grass is also used as material to make soft brooms.

Before climate change, sick animals were just kept under the house and not allowed to roam around. This is to prevent exposure to the elements like rain and heat. Combined with proper care and giving enough food, only a few died and most others survived. With climate change, most of the sick animals die even with extra care given them.

In times of El Nino, they use water from tubogtree (*Ficus nota*) for cooking and drinking. A 15 cm diameter tree can produce two gallons of water. Water can also be obtained when one digs the soil under the cut trunk of cardababanana.

The Bagobo-Klata also continues to nurture the forest as their source of food, lumber, medicine, income and other services. Wild animals that can still be found in their forests are the following: wild pig (*Sus philippensis*), deer (*Rusa marianna*), huge birds like eagles, Philippine civet or milo (*Paradoxurus hermaphroditus*), monkey, and fish. They practice caution in hunting milo so that there will be a continuous supply of this favorite food. The same is practiced for wild pigs and birds. Juvenile animals are not caught and are left to mature for future harvest. For trees, only those big enough to be used for house construction are harvested and the small ones are left to grow for future use.

The Bagobo-Klata have indigenous climate hazard indicator and warning systems. If the temperature is high but the heat is not felt, this indicates that there will be rain in 1-2 months. Atingkarol or kingfisher*(Alcedines*sp.) flying around the village for about five times, signals the coming of the dry season. When the fog or *pamatang*[7]is observed, the arrival of rain is expected.

The Bagobo-Tagabawa Of Davao Del Sur

The Bagobo-Tagabawa resides in Barangay Tibolo, Sta Cruz, Davao del Sur (Figure 3). It is an upland village located 28 km from the town center and has a total land area of 5,713.1612 hectares or 20% of the total land area of the municipality of Sta. Cruz. As of 2000, Tibolo is composed of 212 households with a population of 1,017 and a 4.8 average household size (Municipality of Sta. Cruz, 2013).

The Bagobo-Tagabawa used to be nomadic *kaingin* (slash and burn) farmers. They plant only once and then leave the land to fallow to recover its fertility. Similar to the

[7]*Pamatang* is the fog that covers the lower part of the sun and is observed around 6:00-7:00 in the morning.

Bagobo-Klata, the main source of livelihood of the Bagobo-Tagabawa is farming. Upland rice used to be the major crop planted, often together with corn.

Climate Change and Adaptation of the Bagobo-Tagabawa

The change in climate was observed in the village of Tibolo in 2000. In the past, it was dry and hot in Tibolo when there is typhoon in Luzon. Recently, typhoons also hit the barangay as in Luzon. Heavy rains have been observed to occur more frequently since 2010. This caused the falling off of coffee flowers and non-bearing of fruits of lanzones (*Lansium domesticum*)and other fruit trees. Since 2010 also, there have been more rainy months than dry ones, thus affecting fruit setting resulting to lower yield. The soil has also become waterlogged adversely affecting crop growth and yield.

El Nino or long dry spell of about four to seven months was experienced in Tibolo in 2001. The longest El Nino phenomenon was in 1994, with nine months of dry spell. The drought episodes resulted to total crop failure, particularly of vegetables.

The Bagobo-Tagabawa observed that it is not as cold as it used to be. Before climate change, the weather was generally very cold all throughout the year. Heat intensity now is higher that the forest has receded.

Similar to Bagobo-Klata, the Bagobo-Tagabawa also practice crop diversification in terms of species and varieties as a hedge against crop failure and as sources of additional food and income. Aside from rice[8] and corn[9], vegetables [pole sitao, taro, eggplant, ginger (*Zingiber officinale*), tomato, bell pepper (*Capsicum anuum)*, cabbage, potato (*Solanum tuberosum*), carrot (*Daucus carota* subsp. *sativus*), Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), and Kentucky beans (*Phaseolus vulgaris*)], rootcrops, banana[10], robusta coffee[11] (*Coffea canephora*), coconut[12], and fruit trees [lanzones, marang, durian (*Durio zibethinus*),

[8]Some of the indigenous rice varieties still being planted are *azucena, tubay, kalobkob, habitagan, tipangga, goliath, maya, tulingan, pagimi, mandaya, lipanda, salog, dinorado, and putian*.Since only few farmers are now planting rice,*maya*or rice birds *(Annona reticulate)* havebecome a grave problem during grain filling stage.

[9]Corn varieties being planted are yellow, *senyorita*, *tiniguib*, *pilit*, and *kiyabot. Tiniguib* and *pilit* (glutinous) corn varieties have white kernels while *kiyabot* has red kernel.

[10]*Binangay* and *cardaba* banana varieties

[11]Dried beans are sold in Toril, Davao City, the nearest major market which is about 50 km away

[12]Two types of coconut, *uyog* and *bola*

rambutan (*Nephelium lappaceum*) and mango (*Mangifera indica*) are also planted. Abaca and the different fruit trees were slowly planted in the area to prevent landslides and soil erosion during the rainy season. These fruit trees eventually grew big enough such that the some areas became unsuitable for rice production. Diversity of farm animals is apparent in the presence of carabaos, horses, pigs, goats, and chicken. These are utilized as draft animals or for consumption and selling in the market during lean months. Due to unpredictable weather, some Bagobo-Tagabawa farmers shifted to planting banana, engaging in contract growing of hybrid seed corn seeds (Bioseed), and mortgaging their lands to lowlanders for vegetable production.

The Bagobo-Tagabwa also rely on local knowledge of stars and constellations in rice production. Land preparation and other production practices coincide with the appearance and location of the moon and stars in the night sky. For example, sowing of rice seeds is done during full moon to ensure high yield.

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Figure 1. Map of the Philippines and its neighboring countries (http://twelfthbough.blogspot.com/2010/08/heads-up.html).

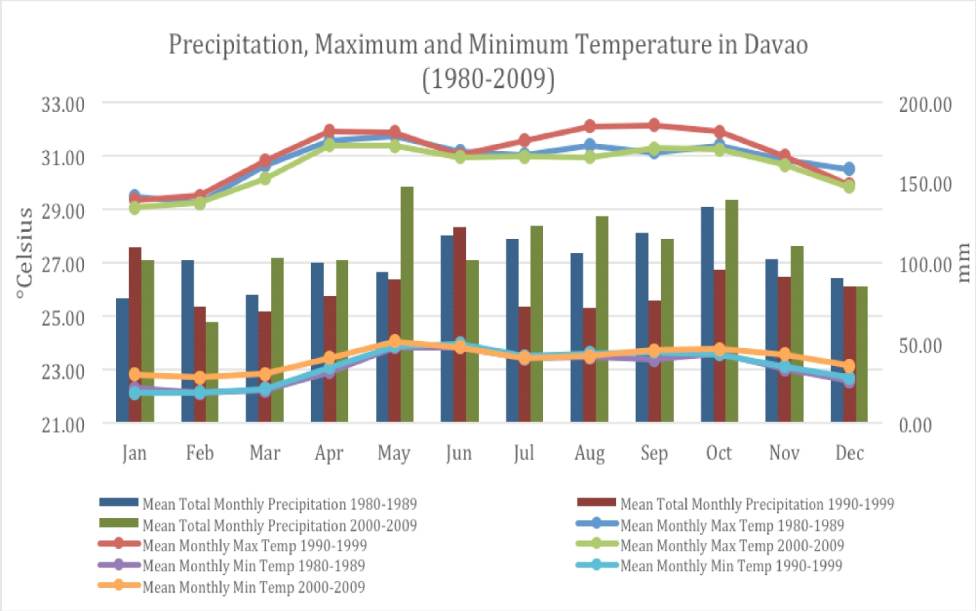

Figure 2. Mean total monthly precipitation and mean monthly maximum and minimum temperature in Davao from 1980 to 2009. Source: http://cfs.ncep.noaa.gov/cfsr

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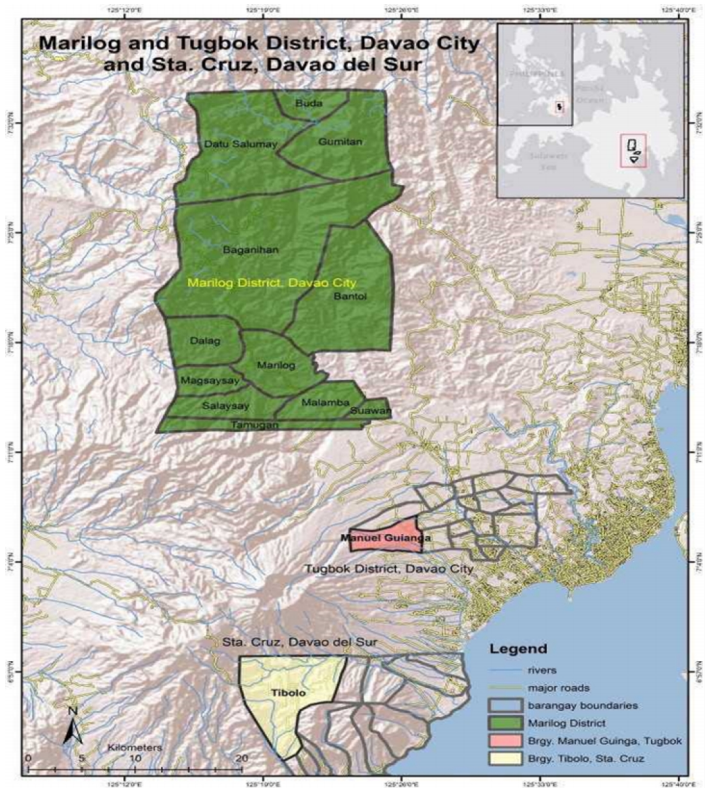

Figure 3. Location map of the Bagobo-Klata of Davao City and the Bagobo-Tagabawa of Davao del Sur.

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MOSAIC DISEASE AND CHILLI PRODUCTION ON DIFFERENT ALTITUDES IN SOUTH SUMATRA, INDONESIA

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ABSTRACT

The study was aimed to investigate the symptoms, severity and effect on yield by mosaic virus disease attack on chilli planted on different altitudes areas in South Sumatra, Indonesia. This study was conducted with survey method and direct observation in the fields. Samples were taken on purposive samplings. The parameters measured were mosaic symptoms and disease severity in the field, identification of virus pathogen with ELISA and chili production at first harvest. From the study it can be concluded that chili in all plantation in three altitudes experienced attack by mosaic virus that is Cucumber Mosaic Virus (CMV) with the most severe was at the low altitude, followed by medium and high altitude; that severity of the disease attack had effect on yield. The highest severity in viral attack had lowest yield of chilli was being at the lowest altitude, although not different from the medium altitude, was significantly different from that of highest altitude of the observed chilli plantation. The discrepancy in the severity is apparently due to the influence of environmental factors. Differences in altitude will cause differences in environmental factors such as temperature, rainfall and humidity can affect a host, pathogen and vector.

Keywords: mosaic virus, disease severity, chili, production, altitudes

INTRODUCTION

Chilli is one of many horticultural crops developed in Indonesia, because the this crop is one of the most high spices use both for household and industrial (Utami, 2011). The average consumption in the country reached 1.074.602 tons with a harvested area of 128.734 hectares and the average productivity reached 8.35 tons per hectare. The chilli

productivity is still low considering the potential of chilli production may reach 10.77 tonnes / hectare (Directorate General of Horticulture, 2015).

The low productivity of chilli in Indonesia is caused by various factors, including the use of low yielding varieties and susceptible to pests and diseases (Kusandriani, 1996). Many organisms attack chilli plants in the field, include mosaic virus attacks that can reduce the production both in quality and quantity.

Mosaic disease caused by a virus is one of the main factors of low productivity in Indonesian chilli (Duriat *et al*., 1995; Suryaningsih *et al*., 1996). As reported by Nyana (2012), a virus that attacks the central chilli plantation in the districts and cities of Bali island is the mosaic virus which caused the leaves to be mosaic and yellowing. Mosaic symptoms associated with three types of viruses namely CMV, ChiVMV, and TMV. Virus infection in many areas of chilli plantations in Indonesia are reported to be high. The symptoms of the diseases in the field vary from mild to striped stripes followed by a severe thickening of the veins (Manzila *et al*., 2011).

The common symptoms of the mosaic diseases in the chilli plantation in Bali is a mosaic symptoms, reaching 57.4% in association with Cucumber mosaic virus (CMV) while yellowing symptoms only 9.2% (Nyana, 2012). In western Sumatra there existed at least four kinds of viruses in the chilli planting area that are Begomovirus, CMV, ChiMV and PMMV (Trisno, 2010). Virus attacks on crops that cause mosaic symptoms will greatly affect crop production, for their mosaic symptoms on leaves cause the decrease of chlorophyll concentrations that can interfere metabolic processes. Study by Sukada *et al*. (2014), showed that the yield of chilli plants infected by the mosaic virus was only 2:52 tons /hectare, while healthy plant with no such disease gives a production of 16 tons / hectare. That describes that the reduction of chilli yield due to viral mosaic infection reached 84, 25%.

The symptoms of the virus in the field are difficult to distinguish, this is caused by several viruses showing the same symptoms. Besides, the variation of these symptoms can also be caused by several factors such as environmental factors, genetic factors, age of the plant by the time plants infected and others. Environmental factors such as geographical differences or altitudes may also affect the development of the disease. This is because of differences in environmental factors such as temperature, humidity, rainfall, amount and duration of rainfall which can affect both plants, pathogen and vector. The interaction

between environment, pathogen and the plant will determine disease severity in plants (Sudiono *et al*, 2005; Jamsari *et al*, 2008).

Controlling of plant viruses is difficult to do because the virus is spread through multiple media such material vegetatively propagated plants, seeds, and insect vectors. In addition, many plant viruses that have a very wide host range (Febria, 2015). The crop damage by the virus in chilli plantation are noteworthy because their viral vector can transfer the virus from diseased plants to healthy plants. Generally the insect vectors of this virus also act as a pest that can cause damage and greater losses. Insects which can be viral vectors e.i. *Bemisia tabaci*, aphids and *Myzus persicae* (Cilia *et al*, 2012).

This research was aimed to study how severe the mosaic virus attacks in chilli plantation in three altitudes namely 500-600 m above sea level (high altitude), 35-67 m asl (medium altitude) and 5-10 m asl (low altitude) in South Sumatra.

MATERIALS AND METHODS

This research used survey method through observations in the field to study how severe the attack by mosaic virus in chilli plants at various altitudes namely 500-600 m above sea level (high altitude), 35-67 m asl (medium altitude) and 5-10 m asl (low altitude). In each region, survey was conducted to observe the incidence of mosaic virus in chili plantation of three farmers's chili farms taken on purposes. Samples in each farmers' chilli farm were taken intentionally (purposive sampling). In each farmer's chilli farm was purposively taken as many as five plots with the size of 5m x 5m. Detection of the virus uses ELISA (enzyme linked immunosorbent assay). The intensity of virus attacks was calculated using the formula by Dolores (1996) as follows:

$$I=\frac{\sum n \cdot v}{N.V} \times 100\%$$

I = Intensity attack symptoms

n = number of plants yan included in a particular symptom scale

v = value of certain symptom score

N = Number of plants observed

V = Value of the highest severity score

Symptom severity scores were classified as follows :

0 = plants not showing symptoms

1 = plants showing symptoms of mild mosaic (1-25 %)

2 = plants showing symptoms of mosaic and yellow groove visible (> 25-50 %)

3 = plants showing symptoms of mosaic and changes in growth (> 50-75 %) .

4 = plants showing symptoms of severe mosaic, deformed growth and dwarf plants (> 75-100 %). . .

Calculated chilli production was the first harvests. The data obtained were gathered and analyzed manually as well as statistically using one-way ANOVA analysis method.

RESULT AND DISCUSSION

The survey results and observations of the disease in the planting area chilli at an altitude of 500- 600 meters above sea level (high altitude), at an altitude of 35-67 meters above sea level (medium altitude), and at a height of 5-10 meters above sea level (low altitude) showed that almost all of the chilli crops infected by mosaic virus. Based on the results of field surveys found that the symptoms of viral disease that attacks the leaves of chilli plants in the form of a mosaic, stunting, leaf narrowing, shriveled and roll (Figure 1) .

The variation of these symptoms may be caused by the influence of environmental factors, genetic plant, the concentration of virus or pathogenic virus strain (Matthew, 1992; Polston and Anderson, 1997). According to Trisno (2010), the low altitude planting area, causing a variety of symptoms of virus attacks, is more complex. The emergence of symptoms is strongly influenced by the strain of virus and other environmental factors such as ambient temperature. Leaf color change will only occur if the ambient temperature above 25ºC and intensity will be increased if the ambient temperature reaches 40 ° C (Dawson, W., 1999).

Detection of the virus in chilli.

The test results for chilli virus with the method (Indirect Elisa) I-ELISA showed that the virus that attack the crop of chili is a single virus namely Cucumber Mosaic Virus (CMV).

Severity of the disease.

The results of the analysis using the method of analysis one-way ANOVA showed significant differences between the severity of the mosaic on the third altitude in the survey (Table 1). Highest severity was found in the chilli plantation with lowest altitude, followed with medium altitude and highest altitude.

Table 1 showed that the mosaic disease severity in areas with altitudes between 500-600 m above sea level was lowest, being 22:15%, significantly different with disease severity mosaic found in chilli planted in area with altitude of 35-67 m above sea level and 5- 10 m asl. The discrepancy in severity is apparently due to the influence of environmental factors. Differences in altitude will cause differences in environmental factors such as temperature, rainfall and humidity can affect a host, pathogen and vector. The state of an ecosystem environment was instrumental in the development of a disease (Nurhayati, 2012). In nature, the spread of the virus through the vector of infected plants to healthy plants is determined by three factors: the host plant, vectors and viral pathogens. The emergence of viral diseases require divulging the conditions include the relationship between vector and host plants as a source of virus in the field and the presence of a vulnerable host, the vector that has the ability to transmit the virus Akin (2006).

The high severity of mosaic disease on chilli plant at the altitude of 5-10 m asl which includes low-lying areas and chilli plant at altitude of 35-67 m asl (medium altitude) allegedly because at this altitude environment suitable both development and distribution of aphids as vectors of mosaic diasease, while on chilli plants at altitude of 500-600 m asl by the time of survey was not found any aphid. The population of *Aphis gossypii* in the chilli plants at the altitude of 5-10 m above sea level was 184 aphids/plant, while in at the altitude of 35-67 m above sea level on there was a number of 49 aphids/ plant. There were no *Aphis gossypii* at altitude of 500-600 m above sea level for by the time of survey was rainy season or high rainfall, while in two other places of ow rainfall. Rainfall at the altitude of 500-600 m above sea level ranges between 2000-3500 mm/year with humidity between 75-89% and a temperature of 14^{o} C- 34^{o}C. Hight incidence of aphids vector on chilli plant might influence by the warm humid climate, vegetation and the presence of secondary host plants for the vector (Fajinmi *et al*, 2011).

Reported that in aphid-borne virus about 5% of the infection on plants occur during the primary spread, the virus pathogens introduced to the plant by vector that pick up the pathogen from an external source such as alternative host, while the remaining 95% of virus infection occurs during the secondary cycle in which the virus spread in the planting area. Vector is the primary means of virus transmission from infected plants to healthy plants. Mulching planting chilli land at an altitude of 500-600 m above sea level is also likely to suppress the presence of viral vectors. Plastic mulch can suppress the population of aphids and reduce the intensity of virus (Uhan and Duriat 1996; Koryati, 2004).

The first production.

The first production of observed plot in the field harvested in three regions of the survey were presented in Table 2.

Table 2 showed that the first harvest chilli mosaic virus infected at an altitude of 500-600 m above sea level at 10.22 g / plant were not significantly different in the production of chilli plants at a height of 35-67 m above sea level but significantly different with the production of chilli in altitude 5-10 m asl, reaching only 7.22 grams /plant. Mosaic virus infection can cause discoloration of leaves (mosaic) such as dark green striped leaves or light green striped leaves, leaf curl, narrow, shrunken, small (dwarf) and necrosis. Symptoms of an attack on the fruit of the fruit shrinking and warping. This is in line with research results by Agrios and Walker (1985), which stated that the chillies that are infected with CMV, has 70-80% small in size fruit that is bent or malformations and pale green in color. Changes in fruit shape or malformations associated with symptoms that occur on the leaves, which resulted in disruption of photosynthesis and inhibits the development of chilli, fruit does not grow normally, causing the decline in the production of chilli.

Viruses replication in infected plants resulting in increased enzyme activity anaplerotik, the rate of photosynthesis and starch content. If the synthesis of viral decline, the rate of photosynthesis and starch content in the leaves will decline, while glycolysis and respiration in mitochondria will increase. These changes are indicated by the occurrence of chlorosis on the leaves (Funayama and Terashima, 2006). Plants infected with the virus will have not normal leafs shape, especially if the plants are infected before entering the generative phase. This will certainly affect the crop leaf area index. If the lower leaf area index and chlorophyll content also lower the amount fotosintat produced for plant growth will also decline. In this

condition, the results fotosintat not only used by plants to grow, but also most of its energy is used by the virus to live and continue to replicate themselves. Net assimilation rate of healthy plants is higher than the net assimilation rate of plants infected with the virus of yellow peppers. This affects the crop production. Chilies produced by healthy plants have the number and weight of higher than the number and weight of the fruit produced by virus-infected plants (Aeni, 2007).

From the study, it can be concluded that chilli in all plantation in three altitudes experienced attack by mosaic virus that is Cucumber Mosaic Virus (CMV) with the most severe was at the lowest altitude, followed by medium and highest altitude; that severity of the disease attack had effect on yield. The highest severity in viral attack had lowest yield of chilli was being at the lowest altitude, although not different from the medium altitude, was significantly different from that of highest altitude of the observed chilli plantation. The discrepancy in severity is apparently due to the influence of environmental factors. Differences in altitude will cause differences in environmental factors such as temperature, rainfall and humidity can affect a host, pathogen and vector.

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a b c

Figure 1. Symptoms mosaic virus attacks in cropping of chili at a height of 500-600 m above sea level (a) , 5-10 m asl (b) 35-67 m asl (c) .

Table 1. Severity of mosaic disease on pepper plants at three altitude

		Std.		95% confidence interval for mean			
Altitutes	Mean severity (%)	Deviation	Std. Error	Lower bound	Upper bound	Min	Max
500-600 m asl	22.153 a	13.362	3.450	14.752	29.554	4.70	57.80
35- 67 m asl	58.13 b	21.489	5.548	46.212	70.013	6.00	70.30
5 – 10 m asl	74.826 b	14.413	4.119	66.844	82.808	43.70	97.90

Table 2. Production of pepper plants infected with mosaic disease on pepper plants at three altitudes

		Std.		95% confidence interval for mean			
Altitutes	Mean (gram/plant)	Deviation	Std. Error	Lower bound	Upper bound	Min	Max
500-600 m asl	10,221 a	1,263	0.380	9.370	11.070	7.50	11.56
35- 67 m asl	9.361 ab	1.878	0.566	8.099	10.623	5.41	12.83
5 – 10 m asl	7.226 b	1.703	0.513	6.081	8.371	4.00	8.75

FARMERS' LEVEL OF AWARENESS ABOUT POLICIES AFFECTING THE HIGHLANDS IN NORTHERN THAILAND

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ABSTRACT

This study seeks to determine awareness of Thai upland rice farmers about environmental conservation policies and sustainable rice farming practices through the promotion of rice terracing in the highlands. The Highland Terrace Paddy Cultivation Technology (HTPCT) was promoted by the Rice Department since 2003 under the Royal Development Project. This paper describes the policies affecting highland terrace paddy cultivation technology and presents the level of awareness of upland farmers about these policies. Secondary data and results from Key Informant Interviews (KIIs) were used to describe the policies affecting HTPCT.A total of 33 adopters and 57 non-adopters of HTPCT served as respondents of the study. Respondents were chosen based on stratified sampling and purposive sampling method.Simple statistical measures were employed to describe and compare both adopters and non-adopters in terms of their level of awareness about environmental conservation policies. Results of the study showed that the dominant polices that sought to promote highland terrace paddy revolved around the need to achieve food security, conserve the forest and promote community based tourism (CBT). Results further showed that the adopters and the non-adopters both had moderate awareness about environmental conservation policies related to land tenure, water and soil conservation, and forest conservation. This implies that farmers give attention in environmental conservation policies but fail to comply with some policies.

Key words: awareness, highland rice terraces, policy study

INTRODUCTION

Background

In Thailand, mountainous landscape is found in the northern part of the country which share border with Myanmar and Lao PDR. Northern Thailand covers an area of 17 million hectares comprising 33 percent of the Kingdom's total land area of 51 million hectares. Agricultural practices in the highland are mainly under rain fed farming system and so-called shifting cultivation (slash and burn agriculture) .Thailand considers shifting cultivation as aggravating river and reservoir sedimentation due to soil erosion which further results to flooding of areas due to the high rates of surface runoff during rainfall events and consequent soil erosion (Punyatrong and Potichan, 2002).

One potential strategy to address problem related to shifting cultivation is to promote terraced paddy fields in the hilly areas. Terraced paddy yields have been found to be almost double those of upland rice, and the cost of converting sloping lands into terrace paddy can be recouped in a few years. While terrace paddy cultivation in upland areas has been in existence for centuries, this system was introduced and promoted in Northern Thailand only in 2003 under the Royal Development Project (Naruebal, 2011). The Highland Terrace Paddy Cultivation Technology (HTPCT) was promoted by the Rice Department in DoiOmpai Mountain areas since 2003. Currently, there are 48 farmers adopting this technology but there are many more who have not adopted the technology Because of this, there is a need to identify the policies that seek to influence the adoption of rice terracing in particular, and those that promote conservation of the highlands in general. Moreover, this study seeks to know the level of awareness of these policies among them. Based on that, this research describes the policies affecting highland terrace paddy cultivation technology and presents the level of awareness of upland farmers about these policies.

METHOD

Study location

The study was conducted in 5 villages; Ban Sadosa, Baan Sam, Baan La Ang Tai, Baan Pak Pai and Baan Mae Laewhich located in DoiOmpai Mountain situated in the district boundary between Mae Cham, Chiang Mai province and Ma La Noi, Mae Hong Son province. The farm lots and residences of the respondents were situated within the elevation

range of 728 meters asl and of 1,465 meters asl.The site has a semi-humid tropical climate with a mean annual rainfall of 1,217.25 millimeters and a mean temperature throughout the year is 27.18 degree Celsius.

Research Design

The study employed correlational research design using both quantitative and qualitative research methods to gather data. For the quantitative part, survey research was employed while for the qualitative part, secondary data and results from Key Informant Interviews (KIIs) were used to describe the policies affecting HTPCT.

Population and Sampling

Respondents come from 5 villages in The Doi-Ompai highland agricultural development station project, Mae Chaem district, Chiang Mai province, Thailand. There are 181 households. To determine the sample population of the study, stratified sampling and purposive sampling method were employed. Yielding 90 respondents composed of 57 non-adopters and 33 adopters.

Table 1. Number of sampling of adopters and non-adopters from each village under The Doi-Ompai Highland Agricultural Development Station Project.

Name Of Villages	Number Of Households	Number Of Non-Adopters	Number Of Sampling Of Non-Adopters	Number Of Adopters	Number Of Sampling Of Adopters
Baan Sadosa	60	48	21	12	8
Baan Sam	38	28	12	10	7
Baan La-Ang Tai	7	4	2	3	2
Baan Pak Pai	29	23	10	6	4
Baan Mae-Lae	47	30	12	17	12
Total	**181**	**133**	**57**	**48**	**33**

RESULTS AND DISCUSSION

Policies Affecting Highland Terrace Paddy Cultivation Technology:

Results of the study showed that the dominant polices that sought to promote highland terrace paddy revolved around the need to achieve food security, conserve the forest and promote community based tourism (CBT).

Policies to achieve food security

Rice has been a staple food source for Thai people. Although Thailand is able to produce enough rice to support the population, vulnerable households such as those from the rural areas cannot meet the daily energy and nutritional needs (Isvilanonda, 2011).Over the past few decades, Thailand has been implementing various agricultural development policies to improve the food security of its population and increase its export earnings simultaneously.

Along this line, Ministry of Agriculture and Cooperative (MOAC) has given priority to food security as highlighted in the Eleventh National Economic and Social Development plan (2012-2016), which provided a strategy for strengthening the agricultural sector, food and energy security. In 2008, the National Food Committee was appointed as the National Food Committee Act 2008 which authorized the Committee to propose a policy and strategy on food security (Thai National Food Committee, 2012).

In 2012, The Thai National Food Committee (TNFC) outlined the strategic framework for food management during 2012-2016 to achieve their vision safe and high quality food for the people of Thailand and the world. This Framework is a Master Plan to support food security (theme 1), food safety (theme 2), food quality (theme 3) and food education (theme 4). TNFC defined 10 strategies under the principle to create food security in Thailand and to manage resources for efficient food production with the participation of all sectors(Thai National Food Committee, 2012). Of the 10 strategies, four strategies, specifically strategy 2, 4, 6 and 9 have direct bearing with highland terrace paddy cultivation technology (HTPCT). These four strategies aim to manage water and land resources for agricultural and community forest, improve food production efficiency, promote food access among household and at the community level and research and develop technologies and innovation at every step of food production. All of these strategies lead to research and development HTPCT to contribute to achieving of food security of Thailand.

Policies to conserve the forest

In 1992, the Cabinet approved a national forest policy to expand the national forest area cover from an estimated 26 percent to a total of 40 percent of the kingdom's land area. This policy was drafted by the National Forest Policy Committee under the 7th National Economic and Social Development Plan (NESDP) (1992-1996).According to this policy, twenty-five percent of the country will be preserved as protection forests for nature conservation, recreation and environmental quality protection, and 15 percent designated as production forest, providing timber and other forest products. Key to accomplishing this goal was the expansion of existing protected areas and gazettal of new national parks and wildlife sanctuaries. A portion of the national forest reserves system, degraded by commercial logging, agriculture and settlement expansion, was to be rehabilitated (ICEM, 2003).

By the year 2000, the North and Northeast regions of Thailand included 31 wildlife sanctuaries and 57 national parks; covering 15.6% of land area defined as strictly protected by the International Union for the Conservation of Nature (IUCN). However, these legal regulations and definitions conflict significantly with the reality on the ground. Protected areas overlap with enclosed large areas of agricultural land where more than 500,000 people live inside national parks and wildlife sanctuaries (Sims, 2010). During the past decades, there has been a considerable increase in the level of national awareness regarding the direct interrelationship between protected area conservation, and the support that it provides for sustainable economic development. This acknowledgement has contributed to a commitment to expand the area of Thailand's protected area system, strengthen the environmental impact assessment process for development projects, and to include protected area management as an integral component of Thailand's National five year Economic and Social Development Plan (NESPD).Stronger recognition of the interrelationship between conservation and development has led to the restructuring of government ministries aimed at enabling improved coordination in natural resource planning, decision making and management for sustainable economic development (ICEM, 2003).

All villages in DoiOmpai involved in the HTPCT study sites are located in the protected areas which are a part of Mae-Cham National Reserve Forest and Mae-Yuam National Reserve Forest.Historically, the villagers in Doi-Ompai practice shifting cultivation regarding to waste of land and leading to a major soil erosion and deterioration (FAO, 2016) of surrounding areas. Moreover, shifting cultivation could not make the villagers meet their

subsistence need. It is for a reason the NESPD was formulated to address the livelihood needs of the inhabitants in the protected areas in order to preserve the integrity of these protected areas.

In 2003, the Doi-Ompai highland agricultural development station (the DOHAD station)was established according to the Queen's speech as one of the royal development projects. Twenty government agencies from Chiang Mai and Mae Hong Son provinces take responsibilities to integrate collaboration for achievement of project which is to protect forest conservation areas along with development of community's livelihood. According to the director of the DOHAD station, three missions have been carried out which are 1) establishment of the DOHAD station, 2) setting up rice bank and 3) setting up firewood bank. The two objectives of the DOHAD station project are 1) to demonstrate alternative crops and test new varieties of vegetables, flowers and temperate fruits for highland and 2) to create working places for villagers that they can engage in farming in the DOHAD station areas and obtain wage per day.

Since the DOHAD station was established, the villagers had the chance to learn agricultural practices by doing and using those knowledge from working in the station for cultivation in their own fields. Villagers were educated about land allocation of protect forest conservation areas which were allocated as areas for farming, community forests and protected forests. They were given information about the areas which are expected to be reforestation areas. The promoting of HTPCT is part of supporting rice bank project of the DOHAD station. According to the Director of DOHAD station, when people have enough rice to consume in their household, they would not clear new areas for rice cultivation and probably they would return the areas that supposed to be forest conservation areas. As the information of DOHAD station, in 2013 the villagers have returned3,685 rai or around 590 hectares for reforestation.

Policies promoting tourism in areas

Thailand envisions to become a tourist's destination. The Thailand National Tourism Development Plan (2012-2016) was proposed by the national tourism policy committee and approved by the cabinet in 2009 to move Thailand to the top five destinations in Asia. One of 5 strategies which have been set for implementation and has direct impact with highland

terrace paddy cultivation technology (HTPCT) is the strategy emphasizing on the development of new product, services and human resource (Wirudchawong, 2012).

Tourism Authority of Thailand (TAT), established in 1959, has played crucial role in tourism development in Thailand. In 1999, TAT promoted agro-tourism and home stay tourism to strengthen community-based tourism. TAT aims at making tourism to be a sustainable form of tourism development. That means tourism resources would be environmentally-friendly, and would be improved with the "Concentration and Attention on Sustainable Tourism". Generally sustainable tourism development projects are referred to as 'alternative tourism' (Scheyvens, 2002).

Community-Based Tourism (CBT) is a form of alternative tourism which aims to include and benefit local communities, particularly indigenous peoples and villagers in the developing countries. CBT is socially sustainable tourism which is initiated and almost always operated exclusively by local and indigenous people. CBT programs are designed based upon special elements of local lifestyle, culture, people and nature that community members feel proud of and choose to share with quests. Benefits of CBT include income, improved hygiene, new skills and knowledge, and funds to support local conservation and social works (Wirudchawong, 2012).

Chiang Mai province is the second largest city of Thailand and it is consistently voted as a popular location for tourism because it is home to interesting and colorful ethnic minorities, known as the hill tribes. Chiang Mai province has several policies promoting CBT as another choice of travelers who love nature and willing to get acquainted with locals, discover their culture and share a moment of their life. An example of a successful terrace paddy tourism site is Baan Mae KlangLuang, Chom Thong district. Mae KlangLuang village became the settlement region of Pga-gan Yaw, a Karenic tribe from Myanmar.One of the distinctive activities to do when visiting here is to appreciate awe-inspiring views of terraced paddy fields, bird-watching and nature trekking which are run and managed by villagers. They also have the homestay services for those who would like to learn about the Karen way of life.

In Mae Chaem district, near the study areas, Pa Pong Peangterrace paddies serves as a jumping point before going to Doi-Ompai (the study site). It is well known as the biggest terrace paddy tourism site in Thailand. Based on interview with the leader of the village, Pa Pong Peangterrace paddies were created around 40-50 years ago by the Karen group

according to indigenous knowledge. They converted slope areas to rice terraces on their own initiative without the promotion from government. Presently, in Pa Pong Peang village, there are eight cottages available for tourists who would like to stay overnight in the community. According to the Deputy District Chief of Mae Chaem district, policies for promoting tourism in Mae Chaem is divided into two; policies promoting tourism for discovering "Thainess" which means Thai culture, local wisdoms, cuisine, handicrafts etc. and policies promoting ago-tourism such as terrace paddies.

For Pa Pong Peangterrace paddies, the district office has proposed policies to support transportation and infrastructure's improvement by integrating budget with other agencies. In fiscal year2016 (October 2015 to September 2016), Provincial Administrative Organization (PAO), Sub-district Administrative Organization (SAO) and District office approved the budget for improving road using to get Pa Pong Peang village. However, the project is not yet operational, due to the fact that these areas belong to Forest National Park and all developmentprograms/projects have to be allowed from the Royal Forest Departmentbefore operating and have to pass the environmental impact assessment (EIAs)to consider theenvironmental consequences(both positive and negative) of the programs/projects. Currently, tourist stay in Mae Chaem city and visit the study site, DoiOmpai, only a day.

Farmers' Level of Awareness about Policies Affecting the Highlands:

Both the adopters and the non-adopters were asked to response to 19questions about policies affecting the highlands which would reflect perspective of the respondents about land tenure, soil and water conservation, and forest conservation.Their responses were rated 1 based on their knowledge and experiences as they have knowledge or experiences in that matter (in the positive perception) and 0 as they do not have that knowledge or experience. For the questions that had a five level of rating; 1 is the highest score for those who do that matter frequently (in the positive behavior expected) and 0 is the lowest score for those who did not do that matter. The total scores were assigned descriptive rating of high awareness (0.68-1.00), moderate awareness (0.67-0.34) and low awareness (0.00-0.33).

Farmers' level of awareness about policies affecting the highland related to land tenure

The respondents were asked six questions about the national law related to the ownership of forest resources, community forest areas that they have, the meaning of

watershed 1-A zone, the land demarcation in each watershed zone, the boundary of watershed class 1A zone that locates near their community and community land title deed.

The overall mean awareness score of the adopters about policies affecting the highlandrelated to land tenure is described as moderate awareness (0.45) similar with that of the non-adopters (0.35). Considered in each questions, both the adopters and the non-adopters had high awareness about the national law related to the ownership of forest resources and the benefits of "community forest". All adopters and almost all (93.0%) of non-adopters answered that they knew that forest resources belong to the Department of National Parks, Wildlife and Plant Conservation (DNP) and Royal Forestry Department (RFD), and that individuals and the private sector cannot own a piece of the land. Likewise, the respondents had high awareness about the benefits of community forest.They have their own community forest which they might use timber and non-timber product from their own community forest. Based on KIIs, community forestry management programs were launched in all villages where RFD and SAOare the host of programs. Currently, Baan Pak Pai has 50 rai (8 ha.) of community forestry, Baan Sadosa has 70 rai (11.2 ha.), Baan Mae Lae has 80 rai (12.8 ha.), Baan Sa Ang Tai has 100 rai (16 ha.) and Baan Sam has 150 rai (24 ha.). However, the respondents do not know much about the meaning of watershed 1-A zone, the land demarcation in each watershed zone, the boundary of watershed class 1A zone that locates near their community and community land title deed.

Farmers' level of awareness about policies affecting the highland related to water and soil conservation

The respondents were asked six questions about the national law related to contaminating water resource, the frequency of cleaning their equipment in stream or river, and the knowledge and experience about check dam, hillside ditch, Vetiver grass and disposing rice straw. Overall, the adopters and the non-adopters had different level of awareness about policies affecting the highland related to water and soil conservation with the adopters having high awareness (0.70) while the non-adopters had moderate awareness (0.66). Considered in each questions, both groups had high awareness with four questions which asked about Wildlife Preservation and Protection Act BE 2535 relates to contaminating water resource, the frequency of cleaning their equipment in stream or river, and the knowledge and experience about check dam, and Vetiver grass. On the other hand, both groups were described as moderate awareness people with the question about how to

dispose rice strawbecause they are not much certain which method is the best way of disposing rice straw whether burning or ploughing into soil. From the farmers' point of view, burning may be seen as the most suitable method of disposing of rice straw. It is not only a cost-effective method but it acts as an effective pest control procedure (Kadam, Forrest and Jacobson, 2000; Dobermann and Fairhurst, 2002).It is also seen as a way of preparing the soil for the next crop as well as releasing nutrients contained in the residue for the next crop cycle (Gadde, et al, 2009). However, burning method is not recommended by the government agencies because it is a cause of air pollutants which is a hazard to people's health(Cheewaphongphang, et. al., 2011). Thus, the best way of disposing rice straw as the recommendation of the government agencies like the Rice Department is ploughing it into soil.

Moreover, both groups had low level of awareness to a question which asked their knowledge about hillside ditch. Most of the respondents do not know what the "hillside ditch" is (the adopters-90.9% and the non-adopters-96.5%). Hillside ditches are shallow ditches built along the contour of hillside slopes which can prevent the flow of water from accumulating as it moves downhill. It can help redirect small amounts of rain water into stable areas, and can break long slopes into shorter segments to intercept surface runoff (USDA, 2016).Thus, the findings reveal that officials from Land Development Department (LDD) need to give more knowledge about hillside ditch to farmers in order to enhance their awareness about water and soil conservation.

Farmers' level of awareness about policies affecting the highland related to forest onservation

The respondents were asked seven questions about the national law related to the prohibition of logging activity, duty of people to do forest conservation, collecting forest products, participation in reforestation activities and makingfire break. The overall mean awareness score of the adopters about policies affecting the highlandrelated to forest conservation was described as high level of awareness (0.82) while that of the non adopters was described as high level of awareness (0.74)as well. Regarding, both adopters and non-adopters had high awareness on prohibition of logging activity, duty of people to do forest conservation, participation in reforestationactivities and making fire break but they had moderate awareness on collecting forest products. However, in terms of practices, some of them are still engaging in logging activity (the adopters-24.2% and the non-adopters-35.5%),

collecting forest products often (the adopters-30.3% and the non-adopters-29.8%) and participating less in reforestation activities (the adopters-72.5% and the non-adopters-100%). Thus, allresponsible agents need to create more awareness of villagers about forest conservation to change the behavior of farmers on such issues.

Overall farmers' level of awareness

As shown in Table 2, the overall awareness score of the adopters had a mean of 0.67 described as moderate awareness people while that of the non-adopters had a mean of 0.59 which is described as moderate awareness people as well. The adopters had the overall scores ranging from 0.38 to 0.89 while the non-adopter had the overall scores ranging from 0.34 to 0.83.

Table 2. Level of farmers' awareness about policies affecting the highlands

Awareness About Environmental Conservation	Adopters (N = 33)		Non-Adopters (N = 57)	
	Mean	SD	Mean	SD
Policies related to land tenure	0.45 (MA)	0.21	0.35 (MA)	0.12
Policies related to water and soil conservation	0.70 (HA)	0.14	0.47 (MA)	0.12
Policies related to forest conservation	0.82 (HA)	0.11	0.74 (HA)	0.15
Overall	**0.67 (MA)**	**0.11**	**0.59 (MA)**	**0.08**
Range	**0.38-0.89**		**0.34-0.83**	

Legend:

Scale Limits	Descriptive Rating
0.68 - 1.00	High awareness (HA)
0.34 - 0.67	Moderate awareness (MA)
0.00 - 0.33	Low awareness (LA)

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CULTIVAR DEVELOPMENT OF CASSAVA AT THE UNIVERSITY OF LAMPUNG INDONESIA

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ABSTRACT

This paper elaborated procedures of cassava breeding and research activities directly and indirectly related to cultivar developments of cassava in Lampung. The objectives of cassava breeding program in The University of Lampung (Unila) are to develop superior cultivars: high yielding, high starch content, suitable for functional rice, suitable for bioethanol, and/or resistant to diseases/pests. Cultivar development of cassava consisted of three main steps, i.e., germplasm enhancement, selection, and yield trials. Germplasm enhancement in this breeding program was conducted by exploration, introduction, and sexual hybridization. Exploration and plant introduction were strarted in 2011 by collecting local clones, superior varieties, and/or breeding lines from East Java (Balitkabi), Central Java, West Java, and Lampung. Germplasms (clones) has been introduced and collected from East Java, Central Java, and Lampung Province; the clones have been evaluated for yield trials and for the parents of sexual hybridization. Diverse F1 populations was obtained as the result of sexual hybridization exemplified by the percentage recombinant type of the color of apical leaves in F1 populations derived from UJ 3, CMM 25-27, and Malang 6 as female parents (50%, 73%, and 73% respectively). Clonal evaluation is being conducted on 1617 F1 plants (clones) in 2016. CMM 97-6 was one of the high yielding clones selected from yield trials; 15 units of prelimenary yield trials are being done in 2016.

Keywords: *Botanical seeds, breeding, clones, Manihot esculenta, sexual hybridization*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in Lampung Province. This province is the largest producer of cassava in Indonesia. Cassava roots can be utilized for food, feed, tapioca, bioplastics, and biofuel/bioethanol (Ceballos *et al*., 2012).

The role of cassava as the source of bioethanol is increasingly important because the Indonesian Goverment plans to substitute 15% fossil fuel with biofuel in 2025. Therefore, the productivity of cassava plants must be increased, i.e., by developing high-yielding cultivars and cultivars with high starch content.

Three important steps of cultivar development of any plant species are creation or enhancement of genetic variation of a population, selection, and yield trials (Utomo, 2012). Enhancement of genetic variation of population that will be the subject of selection can be conducted through plant introduction, germplasm exploration, landraces, sexual and somatic hybridization, genetic mutation, somaclonal variation, and genetic engineering. Selection can be conducted directy or indirecty (marker-asissted selection). Yield trials consist of preliminary and advanced.

The status of cassava breeding in Indonesia and worldwide have been reviewed by Poespodarsono (1992), Jennings and Iglesias (2002), Ceballos *et al*. (2007a), Ceballos *et al*. (2007b), Lebot (2009), Ceballos *et al*. (2010), and Ceballos *et al.* (2012). Because most of cassava cultivars are clones, the breeding procedures to develop superior cultivars are simpler than those of pure lines or hybrids. Clones are phenotypically uniform but genotypically can be heterozygous. In other words, clones can be heterozygous or homozygous; inbreeding to develop homozygous lines is not required.

Cultivar development of cassava in the University of Lampung started in 2011 by collecting and evaluating 40 local clones/landraces from Provinces of Lampung, Central Java, and East Java. Then botanical seeds of cassava were collected and germinated; clones derived from the seeds were evaluated. In 2014, natural and artificial hybridization were started in highland of Sekincau, West Lampung. This paper elaborates studies directly and indirectly related to cultivar development of cassava in Indonesia, especially in Lampung. Some data related to genetic enhancement, selection, and yield trials were presented.

A. Ideotypes and Breeding Objectives

Although breeding objectives depend on the final use of a cultivar, there is some consensus on cassava ideotype (Cock *et al*., 1979; Jennings and Iglesias, 2002; Wargiono *et al.*, 2006; Lebot, 2009). Ideotype is an ideal plant model; a set of ideal characters or traits that should be possessed by a superior cultivar to help the breeders to achieve the breeding objectives (Donald, 1968; Fehr, 1987). The ideotypes of cassava, i.e., high ratio of

roots/stems, harvest index, only one stem per cutting with little or no branching, leaf area index (LAI) between 3 and 3.5, large single leaf area, short internodes and total height of the plant less than 2 m, leaves with long life, approximately eight tuberous roots; short, compact roots, easy to harvest and to peel; quality traits (starch, protein, carotene, low cyanogenic glucosides) (Carvalho *et al.*, 2004); and reduced postharvest deterioration. A cultivar possessing the ideal characters are expected to produce high root yield, high starch content, etc. Other important traits are resistance or tolerance to biotic and abiotic stress.

B. Breeding Methods of Cassava

Most of cassava cultivars are clones propagated vegetatively. Because cassava is naturally cross-pollinated species and selection is mostly conducted at F1 generation, most cassava clones are probably genetically heterozygous (Kawano *et al*., 1978). Cassava plants from the same clone are phenotypically uniform due to vegetative propagation. Because homozygousity is not required, the breeding scheme of cassava is relatively simple (Fig. 1) (Ceballos *et al.,* 2007b; Ojulong *et al*., 2008). Based on Fig. 1, genetic enhancement of a population that will be the subject of selection is conducted by sexual hybridization. Genetic enhancement can also be conducted through plant introduction, germplasm exploration, landraces, somatic hybridization, genetic mutation, somaclonal variation, and genetic engineering. F1 seeds (as a result of sexual hybridization) are germinated and planted; then clonal evaluation is conducted, 1000 – 1500 F1 clones are selected from 3000 – 5000 F1 (Fig. 1). Preliminary yield trial is done at one site with three replications, followed by an advanced yield trial at 2 – 3 sites, with three replications for each site.

Plant introduction is a short-cut procedure in cultivar development (Kasno, 1993). Introduced genotypes or lines can be evaluated or included in yield trial by comparing their performance with standar cultivars. If the performance are better than the standards, an introduced line can be released as a new superior cultivar. Introduced lines with one or more superior characters can also be used as donor parents in sexual hybridization. Poespodarsono (1992) conducted selection of cassava clones introduced from CIAT.

C. Genetic Enhancement through Sexual Hybridization

Cassava is naturally a cross-pollinated species; due to protogyny. Genetic enhancement through sexual hybridization can be done by planting several clones placed randomly in the field, allowing inter-mating among clones through natural or artificial

hybridization. The ability to produce flowers is the prerequisite for sexual hybridization. Our data (unpublished) indicated that the flowering of cassava clones in lowland is much lower than those in highland. Therefore, in this study, 32 F1 clones were planted in Sekincau highland, West Lampung (1000 meter above sea level) in April 2012. Planting distance was 100 x 50 cm; clones were planted randomly. Each row of one clone consisted of 10 plants replicated 2 – 5 times. Each replication included 2 rows of high-yielding cultivars UJ 3. Some clones produced flowers and fruits earlier than 7 months after planting (MAP). Table 1 showed that all 32 clones produced flowers and fruits as late as 7 MAP; the percentage of plants producing fruits ranged from 17% (CMM 25-27-38) to 100% (CMM 25-27-102, CMM 25-27-158, CMM 25-27-167, CMM 25-27-65, CMM 25-27-66, CMM 25-27-92, Klenteng 16, Mulyo 3, dand Mulyo 4). The average number of fruits per plants ranged from 0.5 (CMM-25-27-38) to 33.0 (CMM-25-27-66). These data indicated that it is possible to do inter-mating among clones through natural or artificial sexual hybridization in Sekincau highland, West Lampung.

D. Seed Germination and Clonal Evaluation

The F1 botanical seeds harvested from sexual hybridization or self must be germinated to obtain F1 plants. Study on germination and dormancy of cassava seed is relatively limited. Utomo *et al.* (2012) compared the effects of physical and chemical treatments on the germination rate of botanical seeds of cassava F1 clones derived from female parent cultivar UJ 3 planted in Tegineneng Pesawaran, Lampung in 2011 (Table 2). Seeds were stored in room temperature for 6 months prior to treatment applications. The results of Experiment I indicated that germination rate of seeds submerged in solution of 0.001 M H_2SO_4 were higher than control and other treatments except the treatment of scrubbing using sand paper. In Experiment II, the germination rate of seeds scrubbed with sand paper was 31.3% lower than that of seeds submerged in solution of 0.001 M H_2SO_4. The study on germination and dormancy of cassava botanical seeds is continuing and in progress using seeds harvested in 2015 and 2016. Nearly 3000 botanical seeds harvested from ca.70s clones as female plants in 2015 were germinated and grown on soil medium (10 kg soil in a polybag) in Gunung Terang, Bandar Lampung in December 2015.

One of the objectives of clonal evaluation in this study was to show the level of segregation of F1 population derived from female parents. A study was conducted at Gedong

Meneng, Bandar Lampung in 2012 to evaluate morphological variability of F1 plants derived from UJ 3, CMM 25-27, and Malang 6 as female parents. Botanical seeds were planted in soil medium, then transplanted to the field (50 x 50 cm). Table 3 and 4 indicated morphological variabilities among F1 clones planted at 24 week after planting. Variability among 43 F1 clones derived from UJ 3 as female parents was indicated based on observation of 13 variables (Table 3). The number of leaves ranged from 7 – 44 with the mean of 23; the harvest index ranged from 25.4 – 89.6% with the mean of 54,5%.

Table 4 showed the evidence of segregation or recombination in F1 population. The recombinant types in this study were the F1 clones that were phenotypically different from the female parent. Because The F1 population was half-sib, the male parent could not be identified; the phenotype of F1 could be identical to that of the male parent. The percentages of recombinant type of the color of apical leaves (CAL) in F1 populations derived from UJ 3, CMM 25-27, and Malang 6 as female parents were 50%, 73%, and 73%, respectively (Table 4). The evidence of variability among clones of F1 population may be due to sexual hybridization or selfed of heterozygous female parents. Some of the clones evaluated in 2012 were included in preliminary yield trials in Natar, South Lampung. Some promising clones could not be included in yield trials because the stem cutting dried due to long dry season.

As many as 1617 F1 plants (1617 F1 clones) were obtained from the germination of 3000 botanical seeds. Now, clonal evaluation of the 1617 F1 plants is in progress at the Integrated Research Station, the University of Lampung, Gedong Meneng Campus, Bandar Lampung. Morphological variabilities are observed; In the near future, this will be followed by preliminary yield trials.

E. Yield Trials

A yield trial was conducted in 2011 in Natar South Lampung, using completely-randomized block design consisted of three replications; 40 clones were evaluated. UJ 3 and UJ 5 were used as standard/check. CMM 97-6, CMM 36-5, CMM 38-7, CMM 97-14, CMM 20-2, CMM 1-10, CMM 25-27, CMM 2-8, CMM 36-7, CMM 21-7, CMM 2-2, CMM 2-16, and CMM 21-26 were introduced from Indonesian Legume and Tuber Crops Research Institute, Malang East Java. Duwet- 1, Duwet-3, Duwet-3A, Duwet-4, and Bendo-1 were

introduced from Sragen, Central Java. UJ 3, UJ 5, and Adira 4 are released/standard cultivars; The 19 other clones were collected from Lampung Province.

One of the promising clones was CMM 97-6. Root weight per plot of CMM 97-6 was significanty higher than that of UJ 3 and UJ 5 (Table 5); while the harvest index and starch content among the three clones were not significantly different. The root weight per plot of thirty-three clones were not significantly different from UJ 5. CMM 97-6 and the other promising clones are including in further advanced yield trials.

In 2016, 15 experimental units of preliminary field trial are being conducted in Unila Research Station, Muara Putih Village, Natar, South Lampung. Each experimental unit is arranged in completely randomized block design consisting two replications; 20 – 25 clones are evaluated, UJ 3 and UJ 5 are used as checks.

F. Conclusion

Cultivar development of cassava consists of germplasm enhancement, clonal evaluation and selection, and yield trials. Introduction and collection (exploration) of clones have been conducted from East Java, Central Java, and Lampung Province. The clones have been evaluated in yield trials and for the parents of sexual hybridization. Sexual hybridization produced diverse F1 populations exemplified by the percentage recombinant type of the color of apical leaves in F1 populations derived from UJ 3, CMM 25-27, and Malang 6 as female parents (50%, 73%, and 73%, respectively). In 2016, clonal evaluation is being conducted on 1617 F1 plants (clones). CMM 97-6 was one of the highest yielding clones selected from yield trial; 15 units of prelimenary yield trials are being done in 2016.

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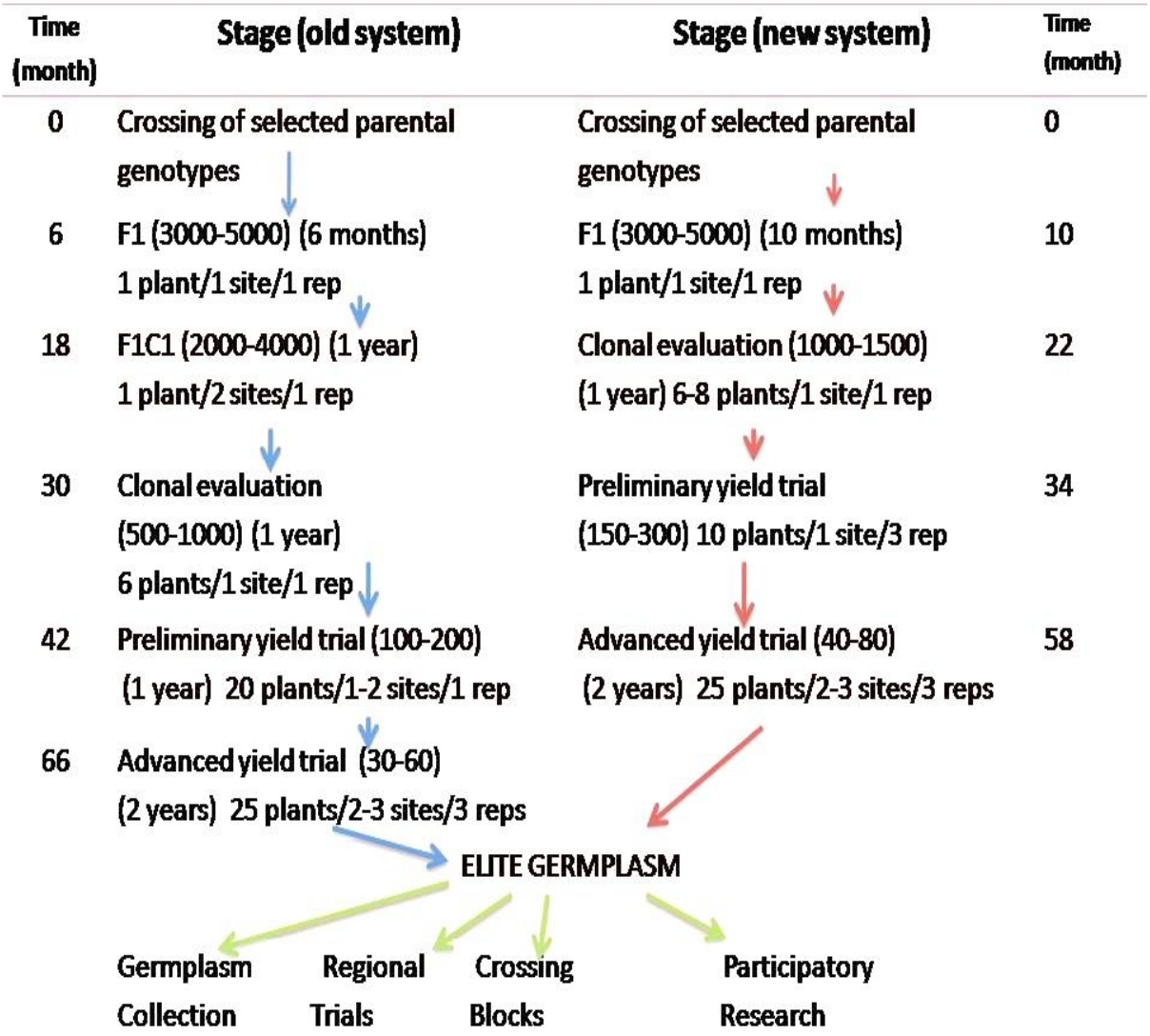

Fig. 1 Scheme of cultivar development of cassava, old (left column) and new system (right)

Source: Ceballos *et al.*, 2007b

Table 1. Number of plants, percentage of plants producing fruits at 7 months after planting, and average number of fruits per plant (cumulative number of three harvest) 32 clones of cassava planted in highland Sekincau West Lampung in April 2012. Clones No. 1 – 13 are F1 clones derived from female parent CMM-25-27; No. 14 – 15 are F1 clones derived from

female parent CMM 97-6; No. 17 – 21 are F1 clones derived from female parent Klenteng ; No. 22 – 23 are F1 clones derived from female parent Malang 6; No. 24 – 26 are F1 clones derived from female parent Mulyo, and No 27-31 are F1 clones derived from female parent UJ 3.

No.	Clones	Number of plants	Percentage of plants producing fruits (%)	Average number of fruit per plants
1	CMM 25-27-102	3	100	5.7
2	CMM 25-27-107	5	80	7.6
3	CMM 25-27-143	11	82	2.4
4	CMM 25-27-145	7	71	19.4
5	CMM 25-27-158	7	100	12.1
6	CMM 25-27-167	9	100	2.4
7	CMM 25-27-38	18	17	0.5
8	CMM 25-27-43	9	22	1.6
9	CMM 25-27-65	1	100	4.0
10	CMM 25-27-66	2	100	33.0
11	CMM 25-27-92	3	100	3.7
12	CMM-25-27-127	20	50	6.3
13	CMM-25-27-42	9	78	5.3
14	CMM 97-6-1	9	89	2.9
15	CMM 97-6-3	7	71	1.4
16	Klenteng	6	33	2.7
17	Klenteng 16	6	100	1.3
18	Klenteng K-16	7	71	9.0
19	Klenteng 17	10	20	1.7
20	Klenteng K-27	3	33	25.7
21	Klenteng-43	3	100	14.0
22	Malang 6-48	5	80	5.0
23	Malang 6-6	12	17	17.8
24	Mulyo-1	16	69	1.1
25	Mulyo-3	7	100	14.2
26	Mulyo-4	7	100	4.0
27	T-12	6	83	6.0
28	T-125	10	60	9.0
29	T-142	15	53	9.4
30	T-19	5	40	12.0
31	T-9-193	4	75	12.0
32	UJ-3	157	62	7.2

Table 2.Germination rate comparison of botanical seeds of F1 cassava clones derived from female parents UJ 3.

Treatment of pre-germination	Germination rate (%)				
	Experiment I			Experiment II	
	Asli	√x			
Control	24,0	4,89	bc	78,7	B
Scrubbed with and paper	41,3	6,40	a	54,7	C
Submerged H2SO4 5 min.	33,3	5,76	ab	96,0	A
Submerged H2SO4 10 min	30,7	5,54	ab	81,3	ab
Submerged KNO3 3%	25,3	5,01	bc	76,0	B
Submergedin water 48 hours	24,0	4,87	bc	76,0	B
Punctured with a needle in the radicle end	21,3	4,59	c	45,3	C
BNJ 0,05	0,89			16,9	

Source: Utomo *et al*. (2012)

Table 3. Morphological variabilities (indicated by minimum and maximum values) of 43 F1 clones derived from UJ 3 as the female parent at 24 weeks after planting.

No.	Variables	Mean	Maximum value	Minimum value
1	Plant height (cm)	60,2	120,9	24,9
2	Petiole length (cm)	17,65	29,6	7,4
3	Leaf-lobe width (cm)	3,24	4,3	1,7
4	Leaf-lobe length (cm)	13,49	22	7,1
5	Leaf number	23,02	44	7
6	Stem diameter (cm)	1,1	2,25	0,57
7	Root diameter (cm)	4,39	5,9	2,2
8	Root lengh (cm)	13,84	18,1	10,6
9	Root number per plant	3,7	6	1
10	Number of non-harvestable roots	2,8	5	0
11	Total weight of plant (g)	1050	2160	200
12	Root weight per plant (g)	540	1280	110
13	Harvest index (%)	54,5	89,6	25,4

Table 4 The diversity of the color of apical leaves (CAL) of F1 clones derived from UJ-3, CMM 25-27, and Malang 6 as female parents. The diversity was indicated by the proportion of recombinant types. The classification of phenotypes was based on the Descriptor of Cassava (Fukuda *et al*., 2010). If CAL was different from that of female parent, the phenotype was recombinant types; otherwise parental.

Color of apical leaves	UJ 3			CMM 25-27			Malang 6		
	Number of plants	Parental or recomb-inant types	Proport-ion (%)	Number of plants	Parental or recombinant types	Proportion (%)	Number of plants	Parental or recombinant types	Proportion (%)
Brown-ish green	12	Recom-binant	28,6	13	Recom-binant	25	9	Recom-binant	34,6
Green	9	Recom-binant	21,4	14	Parental (♀)	27	3	Recom-binant	11,5
Brown	0		0	0		0	4	Recom-binant	15,4
Light brown	0		0	0		0	3	Recom-binant	11,5
Light green	21	Parental (♀)	50,0	25	Recom-binant	48	7	Parental (♀)	27,0
Persen-tase tipe rekom-binan			50,0			73			73

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Table 5. The root weight per plot of 40 clones evaluated in Muara Putih, Natar, South Lampung in 2011. Numbers followed bythe same letters in the same column were not significantly different based on Waller-Duncan multiple range test

No.	Clones	Root weight per plot		
	Orig. (kg)		Transf.√x	
1	CMM 97-6	35.85	5.960	a
2	CMM 38-7	29.5	5.036	abcdefg
3	Malang-6	29.1	5.393	ab
4	CMM 1-10	27.73	5.255	abc
5	UJ 5	27.7	5.185	abcd
6	Melati	26.37	5.130	abcde
7	CMM 2-16	25.7	5.052	abcdefg
8	CMM 36-5	25.67	5.064	abcdef
9	CMM 20-2	25.63	5.060	abcdefg
10	CMM 21-7	25.15	5.015	abcdefg
11	Gayor	24.47	4.930	abcdefg
12	CMM 2-2	23	4.784	abcdefgh
13	Mesa	22.63	4.748	abcdefgh
14	Duwet-3	20.75	4.490	bcdefghi
15	Bogor	19.93	4.464	bcdefghi
16	Adira-4	19.8	4.306	bcdefghi
17	Kasetsarthijau	19.65	4.411	bcdefghi
18	Klenteng	19.63	4.418	bcdefghi
19	CMM 97-14	19.23	4.384	bcdefghi
20	CMM 2-8	19	4.220	bcdefghi
21	Duwet-1	18.9	4.341	bcdefghi
22	CMM 21-26	18.5	4.295	bcdefghi
23	CMM 36-7	18.37	4.178	bcdefghi
24	Garuda	17.8	4.218	bcdefghi
25	UJ 3	17.5	4.177	bcdefghi
26	CMM 25-27	17.3	4.119	bcdefghij
27	Duwet-4	16.6	4.049	bcdefghij
28	Duwet-3A	16.13	3.99	bcdefghij
29	BL-5	15.47	3.929	cdefghij
30	Kasetsart ungu	15.03	3.873	cdefghij
31	Bendo-3	14.97	3.859	cdefghij
32	Bendo-2	14.43	3.778	defghij
33	TM-90	14.4	3.722	efghij
34	Kasetsartputih	13.63	3.671	fghij
35	BL-4	13.23	3.625	ghij
36	BL-1A	11.97	3.393	hij
37	Mulyo	11.95	3.432	hij
38	BL-2	11.3	3.267	ij
39	BL-1	10.8	3.280	ij
40	Bendo-1	7.37	2.713	j
Waller-Duncan 0.05			1.4358	

Table 6. The means of harvest index and starch content of 40 cassava clones evaluated in Muara Putih, Natar, South Lampung in 2011.

No	Clones	Harvest index		Starch content (%)	
1	CMM 97-6	0.620	abcdef	30.600	a
2	CMM 36-5	0.527	defgh	28.133	abcdefghi
3	CMM 38-7	0.584	abcdefgh	25.567	bcdefghijk
4	CMM 97-14	0.600	abcdefg	26.233	abcdefghijk
5	CMM 20-2	0.573	bcdefgh	27.367	abcdefghi
6	CMM 1-10	0.604	abcdefg	27.267	abcdefghi
7	CMM 25-27	0.601	abcdefg	23.167	ijkl
8	CMM 2-8	0.492	gh	28.950	abcde
9	CMM 36-7	0.503	fgh	24.500	efghijkl
10	CMM 21-7	0.599	abcdefg	30.300	abc
11	CMM 2-2	0.548	cdefgh	28.833	abcdef
12	CMM 2-16	0.636	abcde	26.633	abcdefghijk
13	CMM 21-26	0.466	h	26.733	abcdefghij
14	UJ-5	0.604	abcdefg	25.667	abcdefghijk
15	Adira-4	0.602	abcdefg	27.367	abcdefghi
16	Mesa	0.603	abcdefg	26.733	abcdefghij
17	Garuda	0.497	gh	23.900	fghijkl
18	Mulyo	0.643	abcd	24.400	efghijkl
19	Gayor	0.654	abc	25.333	cdefghijkl
20	BL-1	0.508	fgh	24.633	efghijkl
21	UJ 3	0.609	abcdefg	23.433	ghijkl
22	BL-2	0.521	efgh	26.533	abcdefghijk
23	UJ 5	0.630	abcde	27.200	abcdefghi
24	BL-4	0.493	gh	27.233	abcdefghi
25	Malang-6	0.599	abcdefg	30.433	ab
26	TM-90	0.558	bcdefgh	21.733	kl
27	Kasetsart Hijau	0.630	abcde	27.300	abcdefghi
28	Kasetsart Putih	0.603	abcdefg	26.700	abcdefghijk
29	Duwet-3	0.700	a	28.400	abcdefg
30	Bogor	0.660	abc	20.433	l
31	Klenteng	0.585	abcdefgh	28.300	abcdefgh
32	Melati	0.597	abcdefg	24.700	efghijkl
33	Duwet-4	0.569	bcdefgh	21.833	jkl
34	Duwet-1	0.669	ab	29.700	abcd
35	Bendo-2	0.509	fgh	27.467	abcdefghi
36	Bendo-3	0.501	fgh	27.867	abcdefghi
37	Bendo-1	0.563	bcdefgh	25.100	defghijkl
38	BL-5	0.492	gh	23.400	hijkl
39	Duwet-3A	0.578	bcdefgh	30.433	ab
40	BL-1A	0.524	defgh	24.267	efghijkl
Waller-Duncan 0.05		0.12		4.9861	

EVALUATION OF VEGETATIVE AND REPRODUCTIVE CHARACTERS OF F2 GENERATION OF YARD LONG BEANS (*Vigna sinensis* L.) FROM A CROSS BETWEEN A GREEN-SWEET POD AND RED POD PARENTS

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ABSTRACT

High yielding varieties can be obtained from a cross between two parental genotypes. This cross will result in variance in progeny population. This study aimed to look at the variance and broad sense heritability. Eighty individual plants in F_2 population from a cross between a green-sweet pod and red pod parent and the two parental genotypes (20 plants per parent) were planted in experiment station at The University of Lampung, Bandar Lampung, in December 2015. The results showed that the genetic and phenotypic variances of vegetative characters were found to be high in plant height and number of leaves but low in number of of branches. Genetic and phenotypic variances of generative characters were found to be high in number of flowers, number of pods per plant and pod length, but low in flowering date, number of seeds per pod, weight of 100 seeds, the level of crispness, and sweetness. Broad sense heritability all of the characters was high except the character of number of branches in the vegetative phase which was moderate. Two genotypes were found to have ideal characters.

Keywords: crosses, genetic, heritability, variance,

INTRODUCTION

Yard long beans (*Vigna sinensis* L.) have a very high potential to be developed. One way of increasing its production and quality is by breeding which could start with hybridization. Crosses could result in genetic variability the progenies and could be used to produce homozygous expected lines which form the basis for the development of new varieties with novel agronomic characters (Barmawi , 2007). In yard long beans, A cross

between green-pod parents that have stripes on the seed coat and red pod parent is expected to produce superior genotypes with red pods which are sweet and crispy, some important characteristiscs that meet consumers' demand (Ardian *et al.,* 2016)

An F2- generation is the highest segregated population because it consists of dominant and recessive homozygous genotypes, 50 % each. High percentage of heterozygote is derived from two parents that have their respective advantages (Fehr , 1987). This allows to expect a high variability in population which is expected to produce the best individuals out of the population. We expected to obtain superior individuals from a cross between green-sweet-pod and red-pod yard long bean. This study aimed to look at the variance and broad sense heritability and also obtain novel characters of individual progeny.

MATERIALS AND METHODS

Eighty plants of an- F_2 population from crosses between green-sweet pod and red pod parental genotypes and the two parents (20 plants per parent) were grown in experiment station at the University of Lampung, Bandar Lampung, in December 2015. Variables observed were plant height, leaves number, branch number, flowering date, flower number, pod number per plant, pod length, seed number per pod, weight of 100 seeds, and the level of crispiness and sweetness.

RESULTS AND DISCUSSION

In this study, characters that had high variability and high broad sense heritability were plant height, leaves number, flower number, pod number per plant and pod length. The results were consistent with those reported by Sa'diyah *et al.* (2009), Barmawi *et al*. (2013), Ardian *et al.* (2016), Kuswanto (2006), and Jameela (2014). Sa'diyah (2009) reported that in F_3 population of yard long bean derived from a cross between black and striped black testa parents, variables showing high variability and heritability were plant height, leaf number, flower number, and pod length. Jameela (2014) reported that in the F_2 population of *Phaseolus vulgaris* L. from crosses between introduction variety and local varieties, pod number per plant had high genetic variability and heritability. Kuswanto (2006) reported that in the F_2 population of a cross between PS and MLG 15151 yard long bean, pod number, pod weight per plant and harvest age
had high variability and heritability.

High variability allows easier selection for desirable characters. High number of leaves were correlated with high photosynthetic rate in plants. The high rate of photosynthesis would result in accumulated fotosinthate. Fotosinthate accumulation would expectedly increase production and the sweetness of pods. In most plants , the photosynthetic activity of source and the development of sink are in a straight line, in which there is a balance between supply of carbohydrates produced from source (leaves) and filling activity of sink (pods). Sink's filling depends on the process of photosynthesis and carbon status on the organ source. Photosynthesis in source will provide feedback in the form of product accumulation of carbohydrates in the sink (pods) (Mc Cormick et al, 2007).

More number of flower is expected to produce more pods. The more pods are produced, the higher production are reached. A high yield also results from pod length. The higher the variability of pod length pod, the easier selection process is to adjust with consumer tastes. This will be a positive influence on the selling value of yard long beans. According to Budiarti (2011) the size of potential fruit is not necessarily longest or biggest one, but it should also have other characters desired by consumers. According Soetiarso (1996) in Ameriana (1998) the value of household consumer preference towards pod length is moderate (40-60 cm).

Characters of branch number, flowering dates, seed number per pod, weight of 100 seeds, level of crispness and sweetness showed a low variability. All of the characters, except brance number, showed high heritability in the broad sense. Criteria of variability and heritability in a broad sense in the F_2 population from crosses between green pods sweetness x red pods can be seen in Table 1 and Table 2.

Some individual genotypes have been selected on the basis of plant height, leaf number, level of sweetness, pod color, pod length and pod number per plant. Selection of plant height and leaf number was expected to increase the rate of plant physiology (Mc. Cormick, 2007). Selection of level of sweetness, pod color, pod length and pod number per plant was expected to result in high yielding superior genotypes which has red pods. The red color in pod was caused by anthocyanin that act as antioxidants (Ginting, 2011). The individual genotypes exhibiting ideal characters which were selected are genotype numbers 59 and 30 (Table 3).

Table 1. Variability criteria of genotype and phenotype in the F_2 population from a cross between green-sweet pod and red pod parental genotypes of yard long bean.

Character	Genotype variance (σ_g^2)	Standard deviation (σg)	Phenotype variance (σ_f^2)	Standard deviation (σf)	Criteria
Plant height	27778,02	166,67	27910,73	27910,73	High
Number of leaves	73,63	8,58	167,07	167,07	High
Number of branches	1,77	1,33	80,99	80,99	Low
Flowering date	2,59	1,61	3,92	3,92	Low
Number of flower	67.44	8.21	73.04	8.55	High
Number of pods per plant	17.00	4.12	23.65	4.86	High
Pod length	26.64	5.16	29.74	5.45	High
Number of seeds per pod	1.26	1.12	2.21	1.49	Low
Weight of 100 seeds	2.91	1.71	3.81	1.95	Low
Level of crispness	0.18	0.43	0.30	0.55	Low
Level of sweetness	0.16	0.40	0.31	0.56	Low

Table 2. Heritability in a broad sense in the F_2 population from a cross between green-sweet pod and red pod parental genotypes of yard long bean.

Character	Heritability	Criteria
Plant height	0,99	High
Number of leaves	0,91	High
Number of branches	0,48	Intermediate
Flowering date	0,66	High
Number of flower	0.92	High
Number of pods per plant	0.72	High
Pod length	0.89	High
Number of seeds per pod	0.57	High
Weight of 100 seeds	0.76	High
Level of crispness	0.62	High
Level of sweetness	0.52	High

Table 3. Selected individual genotypes in F2 population between green-sweet pod and red pod parental genotypes of yard long bean.

Rank	Genotype number	Character					
		Plant Height	Number of leaves	Level sweetness	Pod color	Number of pods per plant	Pod length
1	59	31	53	5.6	53A	25	67.3
2	30	35	35	5.4	53A	18	58.3

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FLOWER INDUCTION OF CASSAVA (*Manihot esculenta* Crantz) THROUGH THE APPLICATION OF PACLOBUTRAZOL AND KNO_3

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ABSTRACT

Effects of Paclobutrazol and KNO_3 on the induction of Cassava (*Manihot esculenta* Crantz) flowering were observed. Two experiments were set up. In the first experiment paclobutrazol was sprayed through plant leaves in five concentrations consisted of 0, 250, 500, 750, and 1,000 ppm. The second experiment was set up to investigate the effect of paclobutrazol applied through the soil with two concentrations consisted of 0 and 500 ppm and KNO_3 with concentrations of 1%, 2%, and 3% applied through the leaves. The results showed that 500 ppm Paclobutrazol reduced the vegetative growth and it was effective in stimulating flower. Additionally, Paclobutrazol applied through the soil did not affect the flower induction. There is no interaction effect between Paclobutrazol and KNO_3 on the vegetative growth and induction of flower.

Keywords: Flower, induction, KNO_3, paclobutrazol

INTRODUCTION

Cassava in Indonesia, especially in Lampung Province, has been a strategic crop to increase the prosperity of the farmers. Unfortunately, the productivity of cassava per unit area of land is still low which is about 21.4 ton/ha (BPS, 2013). One way to increase the productivity can be reached through breeding program. In most crops, however, yields can be increased through crossing if the flowers of parental plants bloom at the same time. Unfortunately, in low land area the flowers of many clones of cassava appear in different time which brings into the difficulty in crossing the cassava plants to get better genotypes.

Alves (2002) reported that flowering of cassava plant is affected strongly by environmental factors. In some clones flower induction depends on photoperiod of more than 13,5 hours and also related to temperature. Flower initiation of cassava is very important because flower of most clones of cassava needs relatively a long time and most cassava clones do not flower uniformly in time, especially in lowland. Therefore this condition

makes the breeders unable to increase characteristics diversity of cassava plants towards better genotypes of new clones of cassava through crossing among clones.

One of the attemps to get the flower of some crops is the use of plant growth regulator such as paclobutrazol. Some researchers have been successful to induce the flower of some crops by using paclobutrazol such as orange (Poerwanto and Inoue, 1994), mango (Martinez et al., 2008). The mode of action of paclobutrazol is the inhibition of gibberellic acid synthesis in plants. The induction of flower in cassava was indicated by the formation of branches at the end of the primary stem, the number and the size of leaves reduced (Halsey, et al., 2008).

However, the application of paclobutrazol often can cause vegetative and generative apical dormancy. To break the dormancy, Bartolomew and Criley (1988) used KNO_3 10-40 g/l to induce the flower of mango. Assuming that by reducing the plant size, the distribution of the total dry matter to other organs is an important factor to suppress the vegetative growth affecting the induction of cassava flower. Present study was carried out to examine the effects of paclobutrazol and KNO_3 on flower induction in cassava.

MATERIALS AND METHOD

Two experiments were carried out at University of Lampung Research Station with an altitude of 250 m above sea level. The experiments were conducted in August to November 2011. The cassava used was the clone known as Thailand (UJ3). The 25-cm stakes were cut from the 10-month cassava plants. Each stake was planted in a 5-kg black plastic pockets vertically with 1/3 part of it was in the planting media which was the compound of soil and manure (1 : 4). The pockets were arranged randomly with the distance 0.5 m in between. Each plant was fertilized with 10 g Urea, 10 g TSP, and 20 g KCl per pocket. One experimental unit comprised of 2 pockets. Treatment means were separated using the LSD at 5% level of significance.

Both experiments were set up in a complete randomized block design with three replications. In the first experiment the concentrations of paclobutrazol as the factor of treatment consisted of 0 ppm (P0), 250 ppm (P1) , 500 ppm (P2), 750 ppm (P3), and 1.000 ppm (P4). Paclobutrazol was applied through the leaves in three times of applications with 2-week interval at a rate of 20 ml, 30 ml, and 50 ml per plant respectively, to attain full cover spray. The first application was done on 30 DAP.

The second experiment consisted of two factors. The first factor was the application of paclobutrazol consisted of 0 ppm (P0) and 500 ppm (P1) and the second factor was KNO_3 application, consisted of 1% (K1), 2% (K2), and 3% (K3). The treatments were performed on 30 DAP. Paclobutrazol was applicated through the soil with 100 ml per plant once during the experiment while KNO_3 was applied through the leaves in three times of application with 1-week interval at a rate of 20 ml, 30 ml, and 50 ml to spread evenly. Measurements were taken until 120 DAP included plant height, number of leaves, time of flowering, fresh and dry weight of plant.

RESULTS AND DISCUSSION

First Experiment

Paclobutrazol treatment has not affected siginifantly the growth of plant since the first week of application. At the period 90 DAP the characteristics of vegetative growth such as number of leave and stem dry weight were statistically different (Table 1). The data in Table 1 depicted that paclobutrazol had a trend to affect vegetative traits of cassava. Stem dry weight data indicated that there was a reduction of cell division and elongation proportionally with the increasing concentration of paclobutrazol. The data of plant height (Figure 1) show that the non-treated plants were higher than the treated ones, especially at 5 weeks application of paclobutrazol.

Number of leave exhibited a trend of reduction with the increasing dose of paclobutrazol. Plants that were not treated by paclobutrazol showed 12.33 leaves (P0) while plants treated with 1.000 ppm (P4) showed fewer number of leaves i.e., 7.60 leaves. Therefore, it is clear that paclobutrazol had taken its role to inhibit the gibberelic acid synthesis in plants. The results which were not statistically different may be due to the period of observation which was considerably short, i.e., 8 weeks after application.

Physiological changes noticed to form early flowering took place at 9 weeks after planting. All of paclobutrazol treatments could induce to direct the physiological changes to mature condition of plant according to dose of paclobutrazol. Percentage of plants that branched as the first phase of flower induction in 250 ppm paclobutrazol was 16,67%, 500 ppm 66.7%, 750 ppm 66.7%, and 1,000 ppm 16.7%. Among those branches of each level of paclobutrazol the cluster of flower appeared in only 16,67% of branches in 250 ppm and 33.33% in 500 ppm.

The best cluster of flower was noticed in 500 ppm of paclobutrazol (Figure 2). Unfortunately, the cluster of flower was easy to drop off. It may be due to lack of nutrients needed by the flower to grow and develop. The results above gave the evidences that paclobutrazol applied through the leaves has worked to suppress the work of gibberellins acting as flowering inhibitors thereby allowing the flower-promoting factor(s) to work. Flower initiation as the effect of paclobutrazol has been reported by Te-chato (2009), Martinez (2008), and Upreti (2013).

Second Experiment

In the second experiment paclobutrazol applied through the soil alone reduced the vegetative characteristics significantly. Application of KNO3 through the leave did not affect the vegetative variables statistically. The plants treated by paclobutrazol 500 ppm were shorter than those untreated ones (Table 2). Application of paclobutrazol could suppress the height of the plants about half of the height of untreated plants, while KNO_3 with three concentrations had no effect on plant height. The effect of paclobutrazol and KNO_3 were not significant on the number of leave. However, the interaction effect of paclobutrazol and KNO_3 together could affect the stem dry weight (Table 3)

The data in Table 3 depicted that the increase of KNO_3 up to 2% could reduce stem dry weight, at higher concentration the combination with paclobutrazol gave non-significan effect on stem dry weight. On the other hand, paclobutrazol combined with KNO_3 suppressed stem dry weight significantly.

Through the visual performances of the treated plants (Figure 4), the plants treated by paclobutrazol clearly had suppressed the plant growth even though paclobutrazol was combined with KNO_3 up to 3% through the leaves. It may be due to paclobutrazol given through the soil was too high so that the plants had no capability to branch and flower. The application of KNO_3 to all levels of paclobutrazol did not affect the plant development. It is obvious that there was no KNO_3 application with no paclobutrazol that formed bramching at the end of the stem as the beginning of physiological changes of plant development to form flower.

Martinez et al. (2008) observing the effects of paclobutrazol combined with KNO_3 on flower induction in mango precisely found that neither paclobutrazol nor KNO3 alone had a significant effect on flowering. However, in contrast they found that the effect of those

treatments on flowering was seen when paclobutrazol was combined with KNO_3. Other research (Yeshitela, 2013) showed that KNO_3 alone did not give any significant effect on flowering. Significancy was found when it was supplemented by urea.

The application of paclobutrazol of 500 ppm through the leaves suppressed the vegetative growth of the plant and induced the plant to flower. The effect of paclobutrazol was indicated firstly by reduction in vegetative traits of the plant. Paclobutrazol applied through the soil, even though it still inhibited growth of the plant but it had no significant effect on flowering. The application of KNO_3 directed to supplement the negative effect of paclobutrazol had no significant effect on flowering. It is noted that the flower induced by paclobutrazol in this study could not last before the flower opened.

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Table 1. The effects of paclobutrazol application on some vegetative traits of cassava on 90 DAP

Paclobutrazol (ppm)	Number of leave (pc/plant)	Stem dry weight (g/plant)
0(P0)	30.33 b	9.00 a
250(P1)	30.33 b	6.67 b
500(P2)	37.67 a	8.33 ab
750(P3)	24.00 b	8.00 ab
1,000(P4)	32.33 b	8.00 ab
BNT(0.05)	**11.94**	**1.70**

Means followed by the same letters do not differ significantly at 5% level of significance

Table 2. The effect of paclobutrazol and KNO_3 on plant height on 90 DAP and 120 DAP

Paclobutrazol (ppm)	Plant Height (cm)		Stem Dry Weight (g/plant)
	90 DAP	120 DAP	
0 (P0)	24 a	32.2 a	59.2 a
500 (P1)	14.5 b	16.8 b	32.6 b
BNT5%	2.11	3.26	8.6

Means followed by the same letters do not differ significantly at 5% level of significance

Table 3. The effect of paclobutrazol and KNO_3 on the stem dry weight on 120 DAP

KNO_3	Stem Dry Weight (g/plant)	
	Paclobutrazol	
	0 ppm	500 ppm
1%	12.6 a (x)	5.56 a (y)
2%	9.3 b (x)	6.58 a (y)
3%	11.5 ab (x)	6.75 a (y)
BNT 5%	2.2	

Means followed by the same letters vertically and horizontally do not differ significantly at 5% level of significance

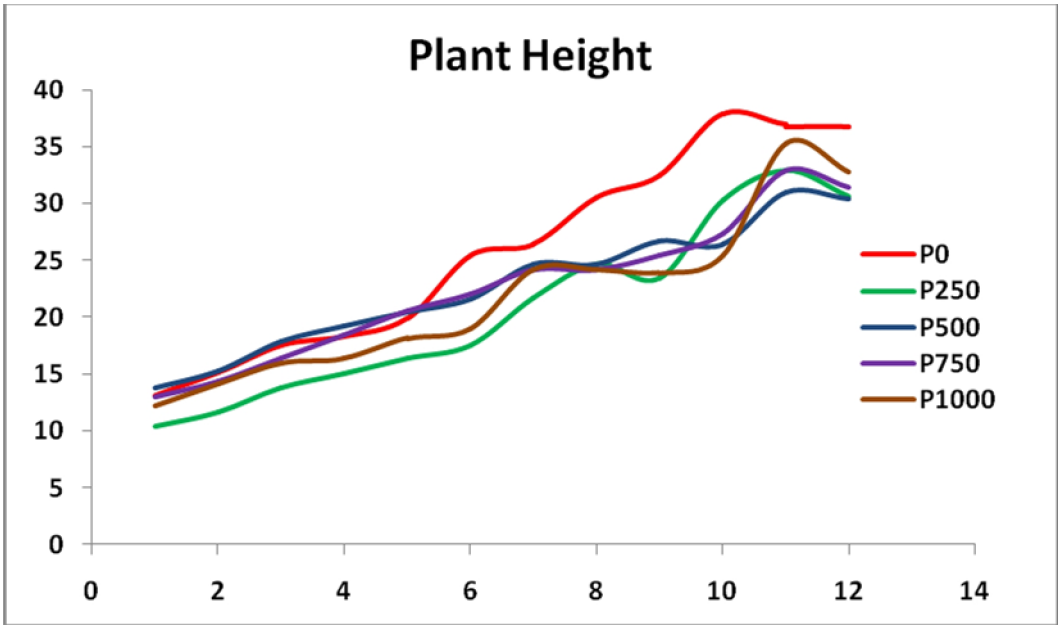

Figure 1. Plant height of cassava treated by paclobutrazol started from one week after application until 12 weeks after application

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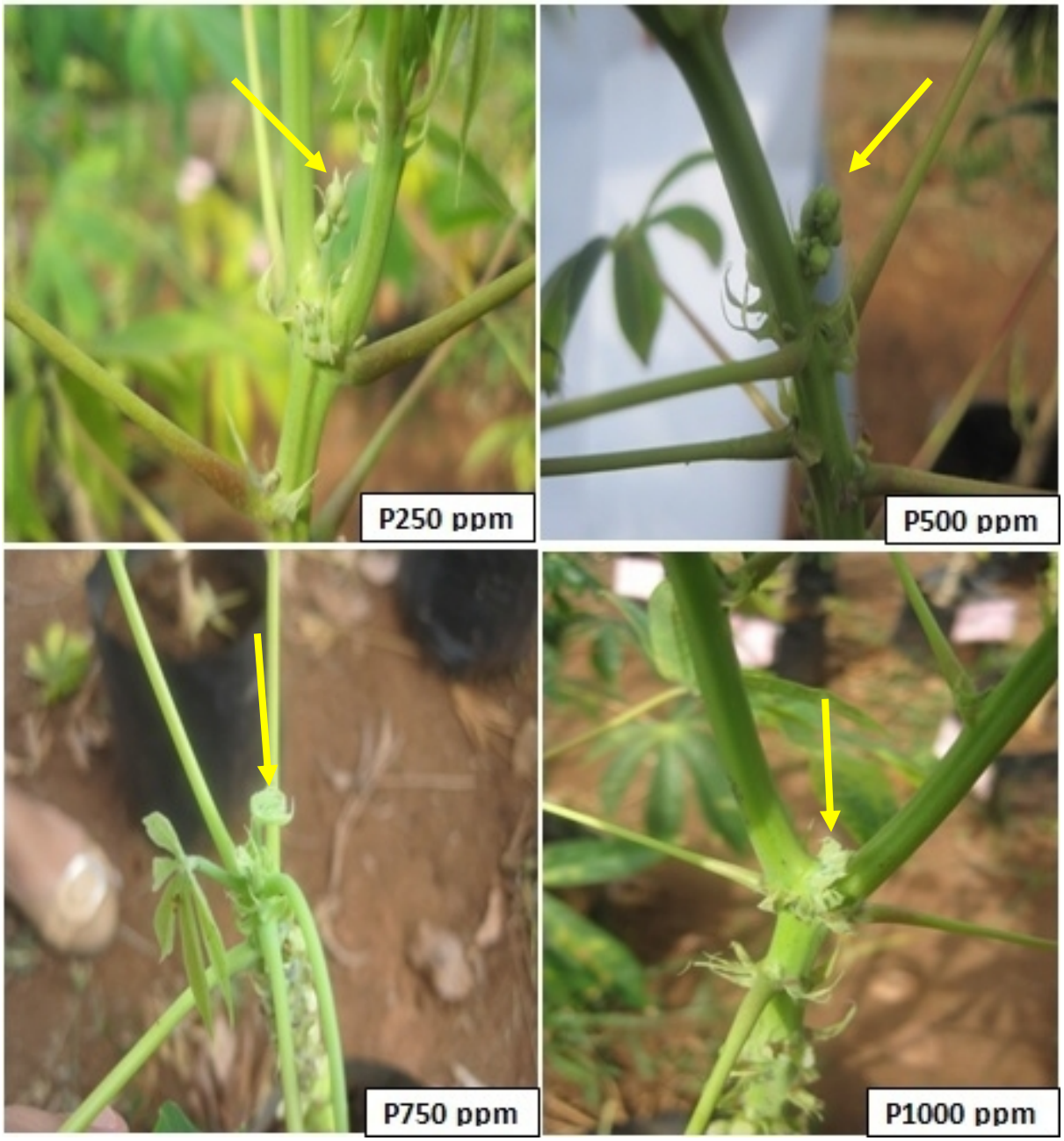

Figure 2. Visual characteristics of plant branching and flowering from each treatment. Branching plants were in treatments 250 ppm, 500 ppm, 750 ppm, and 1.000 Ppm, flowering plants were treated by 250 ppm and 500 ppm (arrows).

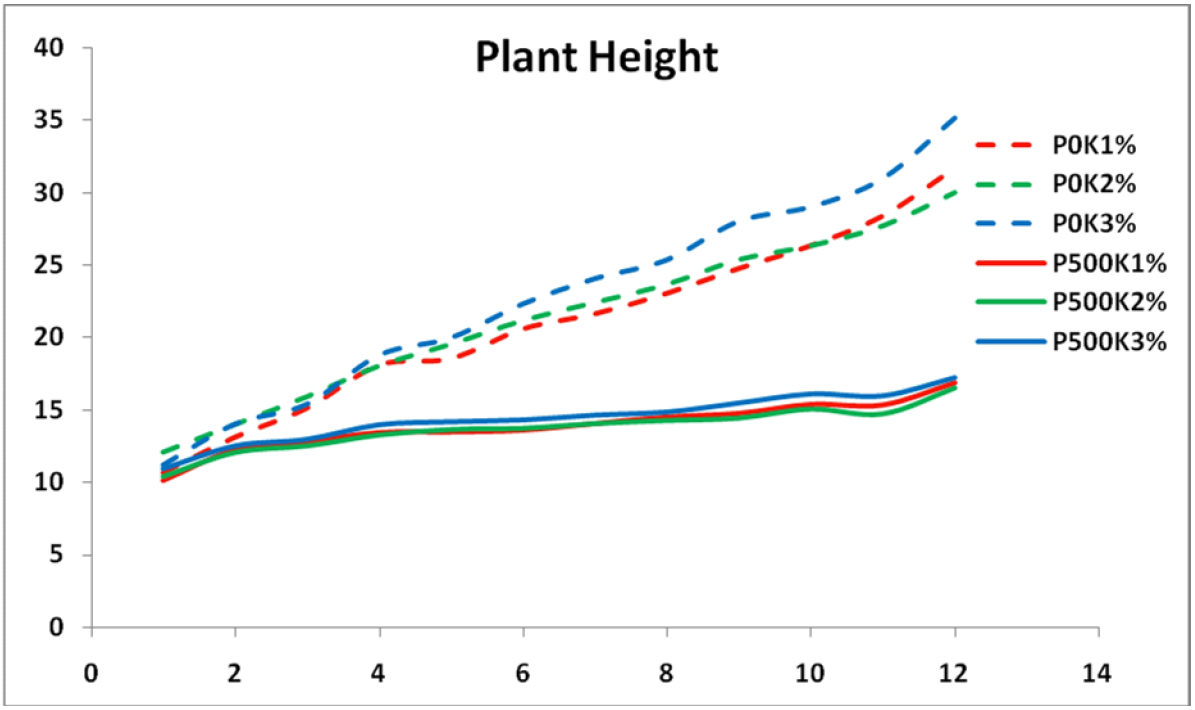

Figure 3. Plant height of cassava treated by paclobutrazol and KNO_3 started from one week after application until 12 weeks after application

ISBN 978-602-0860-08-4

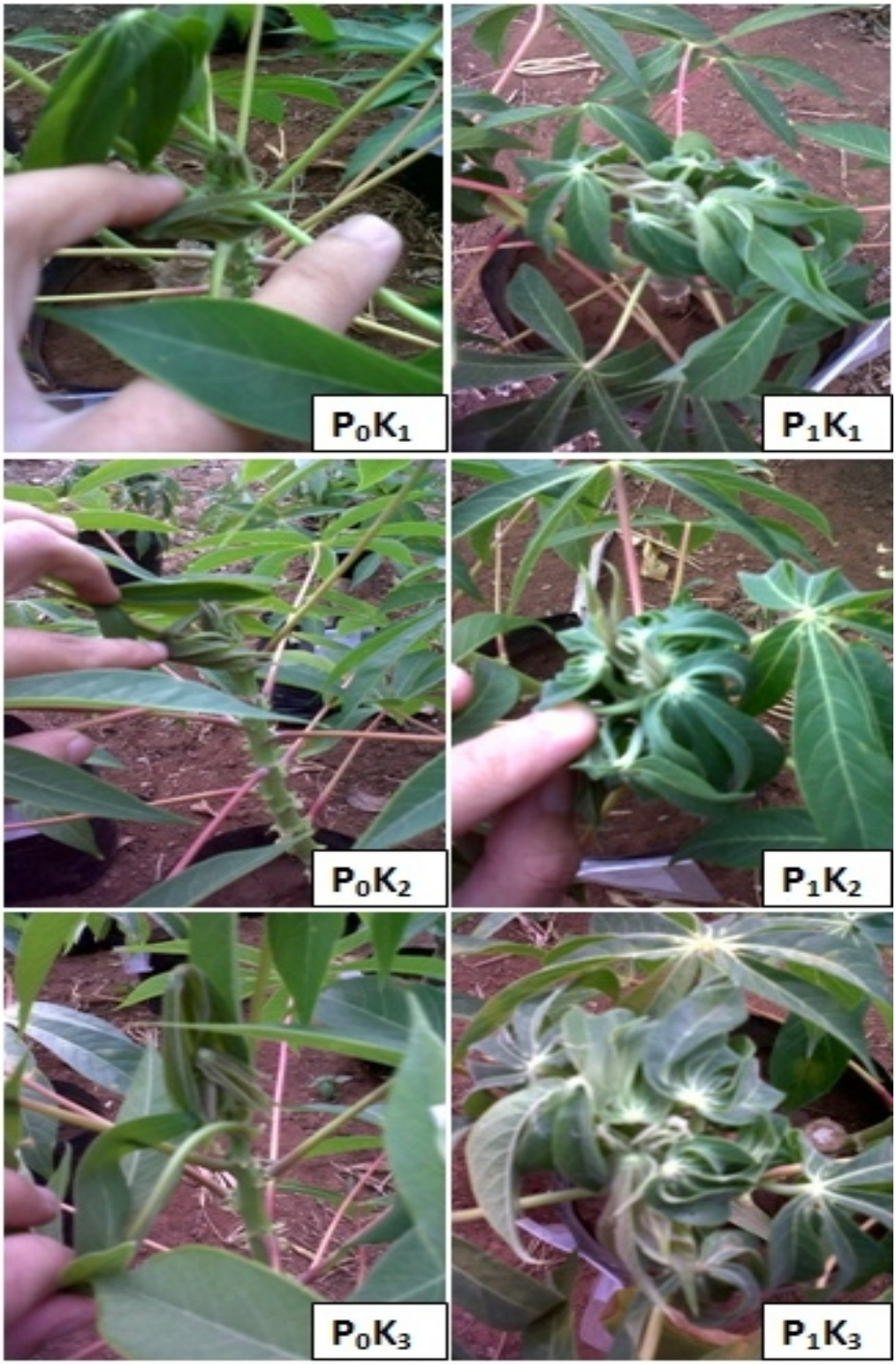

Figure 4. Plant shoots of cassava on 4 weeks after application of paclobutrazol through the soil and KNO3 through the leaves

AGRONOMIC CHARACTERISTICS OF SOME SORGHUM [*Sorghum bicolor* (L.) MOENCH] GENOTYPES UNDER INTERCROPPING WITH CASSAVA

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ABSTRACT

In general, the appearance sorghum agronomic characteristics are believed to be very dynamic, highly dependent on the nature of the genetic and the environment in which plants grow. This study aims to determine the agronomic characteristics of some sorghum [*Sorghum bicolor* (L.) Moench] genotypes under intercropping with cassava. The experiment was arranged in a Split-Plot Randomized Complete Block Design with three replications, in which cropping systems (sorghum monoculture and sorghum-cassava intercropping) as the main plots and 34 sorghum genotypes as subplots. Our results showed that the growth of sorghum were generally not affected by intercropping with cassava, whereas grain yield declined with intercropping as shown by 100-grains weight, seed number and grain weight. There were significant differences among genotypes for all growth and yield components observed. Some genotypes (GH-6, GH-13, P/F 10-90A, P/F 5-193-C, Super–1, Super–2, P/I WHP, Talaga Bodas, UPCA, and Mandau) were able to act equally well as a dual-purpose sorghum to produce above-ground biomass and grain yield.

Keywords: sorghum, intercropping, cassava

INTRODUCTION

Sorghum is one of the most important crops producing cereal. In Africa, sorghum is one of the mainstay cereal crops to fulfill food needs and its cultivation acreage increases every year (Belton and Taylor, 2004). In Asia, sorghum is mainly cultivated in South Asia (Reddy and Patil. 2015). In southeastern United States, beside as forage crop sorghum has been grown traditionally as syrup and sugar crop (Teetor *et al*., 2011). Meanwhile, this crop in Indonesia is not popular due to its low economic value so far, and other factors such as

farmer's knowledge and government's support. In Indonesia, sorghum plantation is still around 8,000 ha spreading in some regions (Supriyanto, 2010).

Cassava is one of the major crops that support the economy of farmers in Lampung Province in addition to palm and rubber. This plant is usually harvested from 9 – 10 months age. The first three months of the beginning of the growth of cassava, the plant canopy still gives open space between rows of cassava, which usually planted with a spacing of 80 cm x 60 cm. Thus, these conditions provide opportunities for intercropping with other plants, such as sorghum. In Lampung, cassava harvested area in 2015 is recorded 279,337 ha (Badan Pusat Statistik Provinsi Lampung, 2016), a huge potential in the use of land for the development of sorghum without having to make major changes in main crop.

Sorghum intercropping systems with cassava has so far rarely been done, at least in Lampung, or if ever has not been well documented. Land use between cassava plants in early its growth will be able to increase the productivity of land, which in turn helps improve the economy of farmers.

Research sorghum intercropped with other crops has also been conducted by researchers, with varying results. For example, other plants that were intercropped with sorghum included pigeonpea (*Cajanus cajan*) (Ito *et al*., 1993), with *Lablab purpureus* L. (Shehu *et al*., 1999), with cowpea (*Vigna unguiculata* L.) (Padi, 2007), soybeans (Ghosh *et al*., 2009), and palisade grass (Borghi *et al*., 2013). Intercropping sorghum – cassava conducted by Kamal *et al*. (2014) shows that planting sorghum 2 or 4 weeks after cassava produce grain yield and nutrient levels lower than planted simultaneously with cassava.

Appearance sorghum agronomic characteristics in general are often regarded as a dynamic, highly dependent on the nature of the genetic (Santos *et al*., 2013) and the environment in which the plants grow. El Naim *et al*. (2012) showed that the sorghum grain yield was positively correlated with the number of grains per panicle and panicle number per unit area. Tolk *et al*. (2013) found that under drought conditions, the stay green hybrid maintained yield by retaining greater seed numbers.

Genotype differences determine the agronomic performance on flowering, dough and physiological maturity phases, while the difference of season gives a slight influence (Munirathinam *et al*., 2013). The big difference in the appearance of agronomic among genotypes can also be caused by physiological differences among genotypes. Djanaguiramana *et al*. (2014), for example, shows the differences in the physiological

appearance among genotypes, in which the plants are tolerant to high temperatures experienced less oxidative damage in leaf and seed pollen than plants that are sensitive. Leaf is part of most plants responsible for photosynthesis. This will have an impact on agronomic performance of sorghum. Research conducted by Sihono (2009), Sihono et al. (2010) and Sihono (2013) also showed variation in agronomic performance of various genotypes tested. In addition there have been improvements in agronomic characteristics of two promising mutant strains that have higher production than the parent, showing that the mutation technique could be one option to improve the agronomic appearance. Elangovan *et al*. (2014) show that genetic diversity among genotypes of sweet sorghum produces the different phenotype that can be viewed from various aspects, both agronomically and biochemistry. Cluster analysis results obtained in this study illustrates the existence of some similarity in traits and yield among the genotypes.

This study aims to determine the agronomic characteristics of some sorghum [*Sorghum bicolor* (L.) Moench] genotypes under intercropping with cassava.

MATERIALS AND METHODS

Time and Experimental Site

Field experiment was started at the end of rainy season of 2015 (rainfall of 186 mm in April) and harvested at dry season (rainfall of 14 mm in September). The experimental site was situated 70 m above sea level on dry land located in Village of Sri Margorahayu, Sub-district of Anak Tuha, Regency of Central Lampung.

Experimental Design

The experiment was arranged in a Split-Plot Randomized Complete Block Design with three replications, in which cropping systems (sorghum monoculture and sorghum-cassava intercropping) as the main plots and 34 sorghum genotypes as subplots. Sorghum genotypes included Mandau, Samurai-1, Samurai-2, Kawali, P/F 5-193-C, P/I WHP, P/I 10-90A, P/I 150-21-A CYMIT, Talaga Bodas, UPCA, Super-1, Super-2, Numbu, Pahat, and 20 mutant sorghum genotypes, namely GH-1, GH-2, GH-3, GH-4, GH-5, GH-6, GH-7, GH-8, GH-9, GH-10, GH-11, GH-12, GH-13, GH-14, GHP-1, GHP-3, GHP-5, GHP-11, GHP-29, and GHP-33. The cassava grown was Variety of Kasetsart.

For sorghum monoculture, each plot consisted of 50 plants grown in a 10 meter-long row, considered as an experimental unit. In sorghum-cassava intercropping, sorghum is planted (at the same time with cassava) between rows of cassava plants. The distance between rows both for sorghum and cassava was 80 cm, while the distance between plants in the row was 20 cm and 60 cm for sorghum and cassava, respectively.

Cultural Practice

Before planting, the soil plowed two times and leveled then plotted. The time span between the first to the second plowing is one week, and leveling the ground was implemented a day after the second plowing.

Application urea on sorghum plants (totally 150 kg / ha) and KCl (100 kg/ha) was done two times that is at 7 days and 30 days after planting (DAP), while SP-36 (75 kg/ha) was applied once at 7 DAP, along with urea and KCl. Fertilization of urea on cassava (totally 150 kg/ha) and KCl (200 kg/ha) was done two times that is at 30 DAP and 90 DAP, while SP-36 (75 kg/ha) was applied once at 30 DAP, along with urea and KCl. Fertilizer is placed in the hole between plants in a row and then covered with soil.

Sorghum was harvested at around 120 DAP, when the seed has reached physiological maturity (varies depending on the genotype).

Data Collection and Analysis

Observations of agronomic characteristics were performed on three samples of plants per experiment unit at harvest. Observations were made on root dry weight, shoot dry weight (leaf + stem), plant height, 100-grains weight, number of grain per plant, and grain weight per plant. The data analysis subjected to Analysis of Variance and LSD, Pearson's Correlation as well by using Minitab Ver.17.

RESULTS AND DISCUSSIONS

Results of analysis of variance for all growth and yield components of 34 sorghum genotypes are presented in Table 1.

The results of our observations showed that there were, except for leaf number at 50 DAP, no significant difference between monoculture and sorghum – cassava intercropping on growth components of sorghum plants. It indicates that sorghum is fairly suitable

intercropped with cassava plant for the forage purpose (Table 1). It is supported by Borghi *et al*. (2013) proved cropping system is very beneficial for both plants intercropped. In this experiment, all genotypes showed significant differences in agronomic performance for all growth and yield components observed. This indicated adequate amount of variability among genotypes that may be helpful for trait improvement by selection as suggested by Khandelwal *et al*. (2015).

Sorghum growth components

The results of our observations suggest that the monoculture system generates the number of leaves (8.3) more than intercropped system (7.6). However, this is not followed by the difference between the two systems for other growth components, such as the dry weights of stem, leaf and canopy. This is an indication that the intercropped system is reliable to produce sorghum forage-based livestock in order to utilize the open space at the beginning of the cassava plant growth.

Some genotypes showed greater growth potential than other genotypes. This is evident from observations at harvest (Table 2) shows that the Super-1, Super-2, and P/I WHP GH-1, GH-2, GH 4, GH-6, GH-13, and P/F 5-193-C tend to be appropriate as forage sorghum. Those genotypes grew taller and had high shoot dry weight. This is in accordance with Wight *et al*. (2012) mentioned that the plant height can be used as a useful indicator of dry mass production in sorghum hybrids sensitive to photoperiod.

Mutant sorghum genotypes of GH-3, GH-5, GH-7, GH-8, GH-9, GH-10, GH-11, GH-12, GH-14, GHP-1, GHP-3, GHP-5, GHP-11, GHP-29, and GHP-33 in this experiment did not show high above-ground biomass.

Sorghum yield components and grain yield

The results showed that the yield components in monoculture system was generally better than intercropped system. This is apparent from the 100-grains weight, grain number, and grain weight per plant of 34 genotypes tested.

Some genotypes like Talaga Bodas, UPCA, GH3, Numbu, Super - 2, GH-6, Mandau, P/I WHP, GH-14, P/F 5-193-C, Super–1, Kawali, P/F 10-90A, and GH-7 produced grain weight higher than the other genotypes (Table 4). This is also supported by the high grain number and 100-grain weight of the genotypes mentioned above.

The differences in the growth and yield among genotypes in this study was similar to the results of Sihono (2009), Sihono et al. (2010) and Sihono (2013) which showed variations in agronomic performance of various genotypes tested. Munirathnam et al. (2013) showed that genotype differences determine the agronomic performance on flowering stage, dough stage, and physiological maturity, while the difference of the season has a little impact. The difference in agronomic appearance among genotypes can also be caused by physiological differences among genotypes.

Grain weight of promising lines of GH-9 and GH-10, and GHP-3 in this study was less than observed by Hadi *et al*. (2016). This difference is probably caused by the location, indicating that those promising lines of sorghum are environmentally dependent.

Based on above ground biomass (revealed by shoot dry weight) and yield (seen from the grain weight per plant) in general there are 15 potential sorghum genotypes as shown in Table 5 below. Ten sorghum genotypes (GH-6, GH-13, P/F 10-90A, P/F 5-193-C, Super–1, Super–2, P/I WHP, Talaga Bodas, UPCA, and Mandau) indicate to be potential as dual-purpose and can be grown for forage or grain production. Forage sorghums are generally taller, leafier and, at least historically, produce less grain than those classified as grain sorghum (Bean *et al*, 2013). Based on Khandelwal et al. (2015) tall plants with high fresh biomass might be poor in translocation of photosynthate, one of the reasons why forage sorghum produces less grain. In contrary, our research showed some promising line (GH-3, GH-6, GH-7, GH-13, and GH-14) were still able to produced fairly high grain although they are tall genotypes. According to Perazzo *et al*. (2014) the negative correlation between plant height and panicle showed that the plant size determines the repartition of the sorghum plant components. Higher plants usually have a higher biomass production and lower participation on the panicles, which becomes a character as forage sorghum. For plants that are shorter, there is a higher percentage of panicles, which becomes a character of grain sorghum. Plants with a medium size has a balanced distribution among the components, as a character dual-purpose sorghum.

The correlation analysis (Table 6) indicates that the root has an important role to above-ground biomass (shown by shoot dry weight) and grain yield.

The results of this study indicate the importance of roots to support stem, shoot, and grain weight. This is evident from the existence of fairly high correlations between root dry weight to stem dry weight, shoot dry weight, and the grain weight. Based on Magalhães *et*

al. (2016), the characteristics of the root has an important role not only in drought avoidance, but also conservative of the water absorbed from the soil.

We can conclude that the growth of sorghum were generally not affected by intercropping with cassava, whereas grain yield declined with intercropping as shown by 100-grains weight, seed number and grain weight. There were significant differences among genotypes for all growth and yield components observed. Some genotypes (GH-6, GH-13, P/F 10-90A, P/F 5-193-C, Super–1, Super–2, P/I WHP, Talaga Bodas, UPCA, and Mandau) were able to act equally well as a dual-purpose sorghum to produce above-ground biomass and grain yield.

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Table 1. Summary of the P values of some growth and yield components of sorghum genotypes intercropped with cassava.

No	Variable	System	Genotype	System*Genotype
1	Leaf number at 50 DAP	0.048	0.000	0.742
2	Plant height	0.162	0.000	0.584
3	Root Dry Weight	0.159	0.000	0.407
4	Stem Dry Weight	0.075	0.000	0.567
5	Leaf Dry Weight	0.106	0.000	0.644
6	Shoot Dry Weight	0.075	0.000	0.563
7	100-grain Weight	0.052	0.000	0.018
8	Grain Number	0.021	0.000	0.613
9	Grain Weight	0.041	0.000	0.298

Table 2. Growth components of some sorghum genotypes at harvest[z]

No	Genotype	Root Dry Weight (g)	Stem Dry Weight (g)	Leaf Dry Weight (g)	Shoot Dry Weight (g)	Plant height (cm)
1	H-1	1.17 ɔc	55.44 ɔcd	17.15 cdefg	72.59 ɔc	198.75 def
2	H-2	8.93 cdefg	52.67 ɔcde	13.56 ghijk	66.23 ɔcde	202.17 def
3	H-3	9.29 cdef	43.82 defgh	14.32 efghij	58.14 defgh	184.50 fgh
4	H-4	1.19 ɔc	51.98 ɔcde	16.89 cdefg	68.88 ɔcd	197.25 def
5	H-5	9.03 cdefg	45.32 cdefgh	15.34 defghi	60.66 cdef	175.00 ghi
6	H-6	9.55 cde	57.07 ɔc	16.31 cdefgh	73.38 ɔc	218.17 cd
7	H-7	8.88 cdefg	44.35 cdefgh	13.55 ghijk	57.90 defghi	186.33 efg
8	H-8	5.65 ghi	30.61 hijkl	10.13 kl	40.74 hijkl	215.58 cde
9	H-9	0.24 cd	34.14 ghijk	11.22 kl	45.36 ghijkl	177.33 ghi
10	H-10	7.97 defgh	44.50 cdefgh	12.27 ijkl	56.77 defghij	192.25 efg
11	H-11	8.81 cdefg	15.00 nn	24.28	39.28 kl	115.83 nno
12	H-12	7.48 defghi	40.94 efghi	9.26 l	50.20 efghijk	159.58 hij
13	H-13	1.21 ɔc	47.74 cdef	17.84 cdef	65.58 ɔcde	152.83 ijk
14	H-14	8.22 defgh	29.41 ijklmn	16.39 cdefgh	45.80 ghijkl	121.92 lmn
15	HP-1	6.18 fghi	14.68 nn	15.43 defghi	30.11	71.11 q
16	HP-3	4.19	13.44	16.17 cdefgh	29.61	86.42 pq
17	HP-5	9.00 cdefg	18.98 lmn	20.56 ɔ	39.54 ijkl	60.00
18	HP-11	4.74 i	17.84 nn	15.83 defgh	33.67 l	71.92 q
19	HP-29	8.56 cdefg	24.88 klmn	18.74 cd	43.62 ghijkl	84.08 pq
20	HP-33	5.41 hi	14.09 ın	18.22 cde	32.31 l	113.00 ınno
21	awali	9.58 cde	30.08 ijklm	18.19 cde	48.28 fghijkl	145.00 klm
22	Iandau	0.03 cd	42.71 defgh	15.48 defghi	58.19 defgh	141.17 klm
23	umbu	7.96 defgh	38.46 fghij	14.09 fghij	52.54 efghij	187.58 efg
24	/F 10-90A	0.78 c	51.08 ɔcde	11.28 kl	62.36 cdef	237.00 b
25	/F 5-193-C /I 150-21-A	0.84 c	56.17 ɔcd	15.58 defghi	71.75 ɔc	240.25 b
26	YMMIT	5.46 hi	25.02 klmn	8.19	33.22 l	194.08 ef
27	/I WHP	0.39 cd	60.37 ɔ	18.83 c	79.21 ɔ	188.08 efg
28	ahat	3.84	15.17 nn	17.47 cdef	32.64 l	99.50 op
29	amurai - 1	7.02 efghi	42.90 defgh	15.54 defghi	58.43 defg	174.67 ghi
30	amurai - 2	5.68 ghi	25.92 klmn	12.87 hijk	38.79 kl	147.75 kl
31	uper - 1	1.99 ɔ	58.48 ɔc	15.30 defghi	73.78 ɔc	229.08 bc
32	uper - 2	4.82	65.86	17.81 cdef	83.66	261.00
33	alaga Bodas	0.73 cd	46.52 cdefg	15.41 defghi	61.92 cdef	190.23 efg
34	PCA	8.79 cdefg	42.79 defgh	17.34 cdefg	60.13 def	173.42 ghij

[z] Means within columns followed by the same letter(s) are not significantly different ($P \leq 0.05$).

Table 3. Performance of vegetative and yield components of sorghum genotypes intercropped with cassava[z]

Components	System	
	Monoculture	Intercropping
Leaf number at 50 DAP	8.25 a	7.55 b
100-grains weight	2.24 a	2.15 b
Grain number	1309.88 a	1063.22 b
Grain weight	30.84 a	24.72 b

[z] Means within rows followed by the same letter are not significantly different ($P \leq 0.05$).

Table 4. Yield components of some sorghum genotypes at harvest[z]

No	Genotype	100-grain Weight (g)		Grain Number		Grain Weight (g)	
1	GH-1	2.37	defg	1108.11	cdefghijkl	24.16	ghijklmn
2	GH-2	2.54	bcde	715.11	kl	18.88	klmn
3	GH-3	2.81	abc	1452.50	bcdef	40.78	abc
4	GH-4	2.37	defg	1033.89	defghijkl	25.72	efghijklm
5	GH-5	2.44	cdef	1120.11	cdefghijkl	27.66	defghijkl
6	GH-6	2.35	defgh	1501.00	abcde	37.78	abcde
7	GH-7	2.54	bcde	1238.89	bcdefghijk	31.80	abcdefghijk
8	GH-8	2.28	defghi	791.39	jkl	18.91	klmn
9	GH-9	2.17	efghij	944.89	fghijkl	21.63	hijklmn
10	GH-10	2.21	efghij	934.83	fghijkl	20.13	jklmn
11	GH-11	1.67	mno	1346.39	bcdefghi	25.74	efghijklm
12	GH-12	2.03	ghijklm	1226.72	bcdefghijk	24.59	fghijklmn
13	GH-13	2.08	fghijk	1234.28	bcdefghijk	29.14	bcdefghijkl
14	GH-14	1.72	klmno	2017.39	a	37.02	abcdefg
15	GHP-1	1.63	no	930.33	fghijkl	16.40	lmn
16	GHP-3	1.94	ijklmn	664.72	l	12.94	mn
17	GHP-5	1.88	jklmn	1011.17	efghijkl	20.46	ijklmn
18	GHP-11	1.78	klmno	892.39	hijkl	17.73	lmn
19	GHP-29	1.21	p	655.89	l	12.43	n
20	GHP-33	1.49	op	835.50	ijkl	16.27	lmn
21	Kawali	2.06	fghijkl	1545.50	abcd	33.51	abcdefghi
22	Mandau	2.36	defgh	1361.06	bcdefghi	37.30	abcdef
23	Numbu	2.87	ab	1363.22	bcdefghi	39.29	abcd
24	P/F 10-90A	2.22	defghij	1431.06	bcdefg	32.79	abcdefghij
25	P/F 5-193-C P/I 150-21-A	2.37	defg	1372.06	bcdefgh	34.20	abcdefgh
26	CYMMIT	1.99	hijklmn	918.39	ghijkl	19.84	jklmn
27	P/I WHP	2.76	abc	1266.06	bcdefghij	37.11	abcdefg
28	Pahat	1.69	lmno	1006.78	efghijkl	17.77	lmn
29	Samurai - 1	2.07	fghijk	1333.83	bcdefghi	28.88	cdefghijkl
30	Samurai - 2	2.21	efghij	1051.94	defghijkl	24.66	fghijklmn
31	Super - 1	2.59	abcd	1231.89	bcdefghijk	34.11	abcdefgh
32	Super - 2	2.50	bcde	1620.56	abc	38.30	abcde
33	Talaga Bodas	2.94	a	1462.39	bcdef	44.33	a
34	UPCA	2.47	cde	1722.39	ab	42.24	ab

[z] Means within columns followed by the same letter(s) are not significantly different ($P \leq 0.05$)

Table 5. Fifteen genotypes of sorghum potential as a producer of livestock and seeds.

No.	Potential as forage sorghums		Potential as a grain sorghum	
	Genotype	Shoot Dry Weight (g)	Genotype	Grain Weight (g)
1.	Super–2	83,6611	Talaga Bodas	44,3278
2.	P/I WHP	79,2056	UPCA	42,2389
3.	Super–1	73,7833	GH-3	40,7833
4.	GH-6	73,3778	Numbu	39,2889
5.	GH-1	72,5944	Super-2	38,3000
6.	P/F 5-193-C	71,7500	GH-6	37,7778
7.	GH-4	68,8778	Mandau	37,3000
8.	GH-2	66,2278	P/I WHP	37,1056
9.	GH-13	65,5833	GH-14	37,0222
10.	P/F 10-90A	62,3556	P/F 5-193-C	34,2000
11.	Talaga Bodas	61,9217	Super-1	34,1111
12.	GH-5	60,6611	Kawali	33,5111
13.	UPCA	60,1333	P/F 10-90A	32,7889
14.	Samurai–1	58,4344	GH-7	31,8000
15.	Mandau	58,1889	GH-13	29,1444

Table 6. Pearson's correlation coefficients between growth and yield components of 34 sorghum genotypes under intercropping with cassava[z]

	Plant Height	Root Dry Weight	Stem Dry Weight	Leaf Dry Weight	Shoot Dry Weight	100-grain Weight	Grain Number
Root Dry Weight	0.373						
	0.000						
Stem Dry Weight	0.602	0.732					
	0.000	0.000					
Leaf Dry Weight	-0.268	0.453	0.188				
	0.000	0.000	0.007				
Shoot Dry Weight	0.499	0.785	0.974	0.404			
	0.000	0.000	0.000	0.000			
100-grain Weight	0.548	0.403	0.568	-0.037	0.520		
	0.000	0.000	0.000	0.595	0.000		
Grain Number	0.198	0.563	0.457	0.375	0.511	0.286	
	0.005	0.000	0.000	0.000	0.000	0.000	
Grain Weight	0.329	0.646	0.616	0.317	0.647	0.545	0.901
	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[z]Number below Pearson correlation coefficient is P-Value

ISOLATION AND CHARACTERIZATION OF INDIGENOUS RHIZOSFER BACTERIA PRODUCING GIBBERELLIN ACID AND INDOLE ACETIC ACID FROM LOCAL SOYBEANS IN SOUTH SULAWESI

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ABSTRACT

This study aimed to isolate and characterize the indigenous rhizosfer bacteria producing Gibberellin Acid and Indole Acetic Acid as plant growth isolated from local soybean of South Sulawesi, Indonesia. Several root samples and soil samples of soybean plants were collected from the rhizosphere of local soybeans in three different areas of South Sulawesi such as Soppeng, Bone and Takalar. There were 56 isolates of bacteria were isolated and grouped into gram-positive bacteria and gram negative bacteria. There are 35 isolates produced a thick slime or slimy when cultured on media Natrium Broth and it was gruoped into gram negative bacterias. The remaining of those produced sporesand clasified as gram positive bacterias. The results showed that of potential bacterial isolated produced Gibbrelin Acid in high concentration. The best isolates of rhizosfer bacteria for the production of Gibberellic Acid are with concentration of 2 %. There are 4 isolates that had higher concentration on producing Indole Acetic Acid is RK 32 (2.794 mg / ml) followed by RK 8 (1.810 mg/ml), RK 23 (1.714 mg / ml), and RK 30 (1.678mg / ml) respectively. The highest isolate producing Gibberelin Acid is RK 30 (4.670 mg/ml), RK 17 (3.797 mg/ml), RK 15 (3.703) and RK 35 (3.222 mg/ml).

Keywords: Indole Acetic Acid, Gibberellin Acid, Rhizosfer Bacteria, Soybeans

INTRODUCTION

Soybean is one of legumes that contain vegetable protein that is high enough range of 34% compared to animal protein (Ditjentan, 2004). Soybeans are not only used as a source of vegetable protein, but also as a functional food to prevent the onset of degenerative diseases, such as coronary heart disease and hypertension. Substance isoflavones contained in soy turns out to function as an antioxidant. Not only that, now soybeans are widely used as

alternative energy sources (biofuels) as a source of vegetable protein, soy is generally consumed in the form of processed products, namely: tofu, tempeh, soy sauce, tauco, soy milk and other forms of snacks (Sudaryanto and Swastika 2007). However, traditional agricultural practices have relied on extensive cultivation of land and the use of pesticides and chemical fertilizers to produce high soybean production. As a result of these, the side effects of this practice have emerged. Some effects include: erosion and loss of topsoil and soil structures damaged from cultivation of the soil, the use of highly hazardous pesticides in the food chain and cause contamination and eutrophication of waters. Therefore there is a need to find solution to solve those problems. One solution can be done in order to solve those problems is to use bacteria as bio fertilizers. According to Klooper et al (1996) the existence of bacteria around the rhizosfer can invade the root system and result in the colonization process in helping the plants to give positive impact and initiate the growth by increasing the plant growth as well as nutrients to the host plants and those bacteria possibly are not synergistic to bacteria and become pathogens. In addition the writer conclude that the potential of those bacteria can also support and increase the many kinds of nutrients such as Nitrogen, Phosphate and also performing the plant growth hormone.

One of the plant growth hormone can be produce by the rhizospheres bacteria is Gibberellin Acid. It is one of hormone promoting the growth plant economically and industrially. As hormone plant growth Gibberellin Acid can be produced from by higher plant around the rhizosfer area by bacteria (Pandya and Desay, 2014). As common structural of the Gibberellin is tetracyclic diterpenoid acids which are produced in the growth and metabolism process of the plants , such as at the germination process of seed, promoting seedling process, leaf as well as stem of plant growth. (Bottini, Cassan, Piccoli, 2004). The aim of the study is isolate and characterize the ability physiologically of rhizosphere bacteria isolated from local soybeans in South Sulawesi potentially as bio stimulant and bio fertilizer which can be applied to the plants and confer protection against soil borne pathogens.

MATERIALS AND METHODS

Source of Bacteria Site

Soil samples have been taken at a depth of 0-20 cm in the three area of soybeans rhizosphere in South Sulawesi, Indonesia. In each area, one healthy soybean were

taken and placed in the plastic bag for isolation process. The samples of soil around the rhizosphere area were collected in one bag and labeled those with the criteria of the site where the samples have been collected.

Samples of Isolation from rhizosphere soybeans

Isolation of rhizosphere bacteria carried out by a serial dilution method. Ten grams of rhizosphere soil was weighed and dissolved in 90 ml of sterile water, then shake for 30 minutes. One ml of rhizosphere soil suspension was added to a reaction tube containing 9 ml of sterile water to get suspension with 10^{-2} concentration of the dilution. The same process was done for several level of concentration such as 10^{-3}, 10^{-4}, and then 10^{-6} respectively. The next step is took 0.1 ml of the suspension and it was grown on media specific such as Yeast Mannitol Agar and Tap Water Yeast Extract in a petri dish Both medium which already contains rhizosphere bacteria were incubated for 24 hours at room temperature. Every single colony were grown to reisolate and made as pure culture and planted on the Nutrient Agar media 10^{-8} suspension. Subsequently 0.1 ml of the suspension was grown on NA medium in a petri dish. NA medium which already contains rhizosphere bacteria were incubated for 24 hours at room temperature. Every single colonies were grown to reisolate and made as pure culture.

Gibberellic acid (GA3)

The production of Gibberellic acid was tested by culturing the bacteria on to nutrient broth media (Borrow et al, 1995). Furthermore one ml of bacterial isolates were added to the media and incubated at 37˚C for seven days. The cultures then were centrifuged at 8000 g for 10 min to remove the bacterial cells. Fifteen cultures were add to 5 ml of zinc acetate. Account after 2 minutes was added 2 ml of potassium Ferro cyanide solution and centrifuged at 8000 g for 10 min. Five ml of the supernatant was added to five ml of 30 per cent hydrocloric acid and the mixture was incubated at 20 ^{0}C for 75 minutes. The blank cuvette was prepared with five percent hydrocloric acid. Absorbance was measured at 254 nm in the UV- VIS spectrophotometer. From a standard curve prepared by using gibberellic acid solution of known quantities, produced of GA by the culture was calculated and expressed as mg l^{-1}.

Indole acetic acid (IAA)

Production of indole-3-acetic acid (IAA) by bacteria was tested using nutrient broth and Salkowski reagent (Gutierrez et al, 2009). PGPR isolates cultured in NB is equipped with L-tryptophan (0,1g l^{-1}) at room temperature in the dark for five days, and the supernatant was taken after centrifugation. One ml of the supernatant was added to one ml of Salkowski reagent (12 g l^{-1} $FeCl_3$ in 429 ml l^{-1} H_2SO_4) (Glickman et al., 1995) and incubated in dark for 24 hours at room temperature. The intensity of pink color developed was read at 535 nm using a UV-VIS spectrophotometer. From a standard curve prepared with known concentration of IAA, the quantity in the culture filtrate was determined and expressed as mg l^{-1}.

RESULTS AND DISCUSSION

Our result showed that the rhizosphere bacteria isolated from three different areas of south Sulawesi is rhizosphere bacteria producing plant growth and development is also regulated by phytohormone. The production of phytohormones such as Gibberelic acid and Indole Acetic Acid which produced by PGPR's have been reported by many researchers, however not many evidence regarding production of gibberellins by the plant growth promoting rhizobacteria has been reported (Amar et al.,2013). Yet, it has been reported to be produced by certain rhizosphere bacteria's such as Brady rhizobium japonicum (Boiero et al, 2007). Gibberellins also can alter the plant morphology by the elongation of stem tissues. (Amar et al., 2013). The results showed in the figure one presented the source of isolates producing Gibberellic acid and Indole Acetic Acid. The highest number of isolates was successfully isolated from rhizospheres from Soil in Soppeng area followed by Takalar and then Bone. The isolated of root rhizosphere mainly from Bone and then Soppeng. None of the isolated can be isolated from the Takalar area.

Indole Acetic Acid (IAA) Produced By Rhizosphere Microbes

The ability of the bacterial isolates to produce IAA was detected by the development of pink color after the addition of salkowski reagent to the culture. Some species of bacteria have the ability to produce IAA. Many evidences suggest that PGPR can affect plant growth and development as it can produce phytohormones. Phytohormones such as auxin (IAA) is known to stimulate cell elongation and cell division differentiation (Achmad et al., 2005),

and gene regulation (Ryu et al., 2008). Indole acetic acid is the common natural auxin that shows all auxin activity and extensively affects plants physiology (Etesemi et al., 2009). Indole acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement (Glick et al., 1995). In our study, all 35 isolate were able to produce IAA growing in medium addition of tryptophan. Maximum IAA production was recorded in isolate RK32 (2.794 mg l^{-1}) as compared to other isolates. The minimum amount of IAA production was recorded in RK 4 (0.190 mg l^{-1}).

Gibberellic Acid Produced by Rhizozphere Microbes

The production of Indole Acetic Acid and Gibberellic has been analyzed on the several isolates which isolated from rhizozphere area of soybean in several district areas of soybean plantation in South Sulawesi. It was found that there 35 isolates identified as gram negative bacteria can produced Indole Acetic Acid and Gibbrellic Acid as well. The highest production of Indole Acetic Acid was produced by the isolates RK 32 (2.794 mg/L) followed by RK 1 (1.714 mg/L) and then RK 23 (1.698 mg/L). The production of Gibbrellic acid also was investigated on the several isolated microbes. The finding showed that the highest number rhizozphere bacteria produced Gibbrellic acid is RK 30 (4.670 mg/L) and followed by RK 35 (3.222 mg/L) and the last is RK 17 (3.797 mg/L). The capabilities in producing the plant growth hormone indicated that those bacteria are good to use in plant to improve the growth and production of plant. According to Bottini, Cassan, Piccoli (2004) discuss Gibberellin can improve the growth and metabolism process of the plants , such as at the germination process of seed, promoting seedling process, leaf as well as stem of plant growth.

Bacterial isolated from rhizosphere of local soybean of several districts of South Sulawesi had more than one physiological character. Isolates RK 32 produced the highest level of IAA concentration, and the isolates RK 30 produced the highest number of GA concentration.

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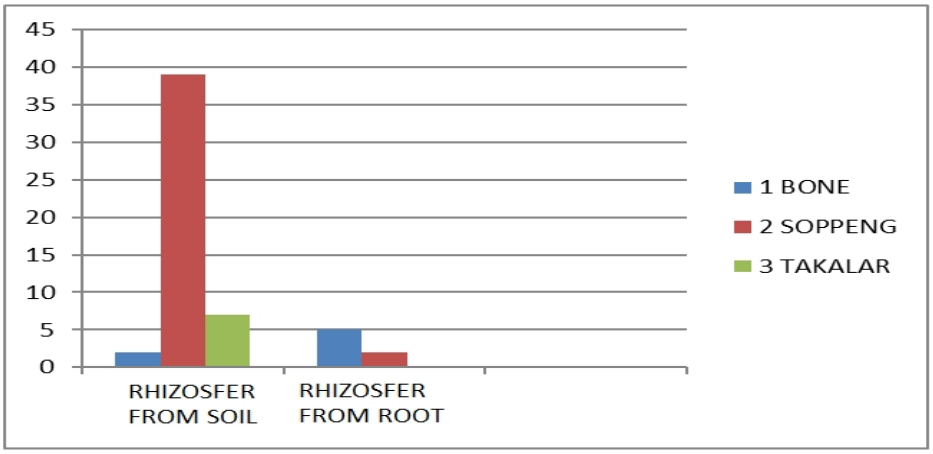


Figure 1. Sources of Isolates Producing Gibberellin and Indole Acetic Acid (IAA)

Table 1. Level of concentration Isolates Producing IAA and Gibbrellin Acid

No.	Isolates	Concentration of IAA (mg l^{-1})	Concentration of GA (mg l^{-1})
1	RK1	1.714	2.902
2	RK2	0.651	2.512
3	RK3	0.698	2.389
4	RK4	0.190	2.628
5	RK 5	1.540	2.573
6	RK 6	1.365	2.539
7	RK 7	0.921	2.687
8	RK 8	0.968	2.455
9	RK 9	0.571	2.521
10	RK 10	0.571	2.858
11	RK 11	0.714	2.949
12	RK 12	0.328	2.439
13	RK 13	0.413	2.535
14	RK 14	0.937	2.856
15	RK 15	1.333	2.645
16	RK 16	0.889	2.743
17	RK 17	1.095	3.797
18	RK 18	0.905	3.176
19	RK 19	0.683	2.628
20	RK 20	0.365	2.588
21	RK 21	0.889	2.538
22	RK 22	0.968	3.135
23	RK 23	1.698	2.622
24	RK 24	0.635	3.293
25	RK 25	1.079	3.703
26	RK 26	0.857	2.419
27	RK 27	0.302	2.502
28	RK 28	0.841	2.698
29	RK 29	0.730	2.658
30	RK 30	1.429	4.670
31	RK 31	1.222	2.654
32	RK 32	2.794	2.476
33	RK 33	0.429	2.571
34	RK 34	0.825	2.588
35	RK 35	0.638	3.222

ESTIMATION OF METHANE(CH_4) EMISSION BASED ON PADDY HARVEST AREA IN LAMPUNG PROVINCE, INDONESIA

ONNYCHRISNA P. PRADANA[1],TUMIAR K. MANIK[2] AND WARSONO[3]

ABSTRACT

Contribution of greenhouse gas (GHG) emissions from agriculture sector comes from paddy cultivation system, flooded paddy field is one source of methanee emissions.Severalresearchs related to greenhouse gas emissions from agriculture (paddy field) by direct measurements have been done in plot scale. This research aimed to predict methane annual emissions from paddy field in regional scale (province) based on paddy harvest area. Method that used in this research are IPCC model to estimate the emission and time series method (ARIMA) to forecast the emission in next five years. The result of this research shows: (1) methane emissions from paddy field are predicted to decline in the next five years, the number will be 232.703 Gg (2013); 229.113 Gg (2014); 225.877 Gg (2015); 222.961 (2016) and 220.333 (2017) for methanee emissions; (2) comparing the result from other country with similar area, it can be concluded that IPCC model could be applied to estimate methane emissions in Lampung; and (3) the amount of methane annual emissions from paddy field is effected by annual cultivated area/harvested area and cultivation period.

Key words:emission, methane,ARIMA, paddy field, IPCC.

INTRODUCTION

Climate change phenomenon as a result of global warming is a real earth problem that affects people life. The global average surface temperature of the Earth has increased by 0.6± 0.2 ^{o}C since 1900 and it is likely that the rate and duration of the warming are greater than at any time in the past 1000 years (IPCC, 2001).Earth temperature rise because the longwave radiation emits by the earth surface is trapped by certain gasses known as greenhouse gasses.

Global warming will affect climate processes and feedbacks and result in changes of mean temperature and precipitationdistributions and is also expected to affect interannual and longer time-scale of precipitation (Boer, 2009). For instance monsoon rainfall over South Asia has decreased during the last 5 to 6 decadesaccording to several sets of observations (Annamalai *et al.*, 2013). Global warming induces increased frequency orintensity of

typhoons; for that reason, coastal zones and riversides are considered to be the residential areas that are most likely to be influenced by global warming (Yasuhara*et al.*, 2010).

One of the greenhouse gases that play a role in global warming is methane. Methane (CH_4) has Global Warming Potential (GWP) value 21 times greater than CO_2 (ASA et al., 2010). Concentration level of methane has reportedly increased from 700 ppb in 1750 became 1774 ppb in 2005 (Hidore et al., 2010).

Methane is producedin an anaerobic environment such as rice paddies, swamps, sludge digester, rumens and sediments (Yang and Chang, 2001). Rice paddies have been identified as a major source of atmospheric CH_4. Global annual methane emission from rice fields were estimated to range from 25—100Tg which contributed to 10—30% of global methane emission (Yang and Chang, 2001).Various researches related to direct measurement of greenhouse gass emossion from paddy field have been done in plot scale.Next step is toupscale the results to for example city or regional scale. Since direct measurements might not available in this scale, models could be used to for this purpose usingdata from direct measurements from the plot scale.

Model is a development of equations that describe the relationship between certain variable, parameters, management or control input and environment input (Jones and Boote, 2007). IPCC (*International Panel for Climate Change*) has developed some mathematics models to predictmethane gas emission from paddy field(IPCC, 2006).

Besides upscalling the direct measurement to larger scale, it is also needed to forecast the methane emission in the future years. There are two methods in forecasting: qualitative and quantitative. In quantitative there are causal/regresion method and timeseries methods. Time series method is used when the intention is only to forecast future results without analyzing the process. In time series, Box Jekinsmethod could be a main alternative if inside the data exists some complicated data pattern(Makridakis*et al.*, 1999).In Indonesia, Box Jenkin method for agriculture application has been done to forecast sugar cane production Kristyawan (2003) and Istiqomah (2006).In Climatology, Box Jenkin has been used to forecast rainfall anomaly based on monsoon index and El Nino event (Edwuard, 2013).

Because rice is the main staple food in Indonesia and methane emission is crucial related to global warming, it is necessary to forecast its future emission using a suitable model based on paddy harvest area in Lampung Province, Indonesia.The objectives on this research is to forecast annual methane emission in five years period (2013—2017)in

Lampung Province based on paddy field area in the last 20 years (1993—2012). From this research it is expected that methane emission in this province could be quantified that agriculture project plan should consider mitigation effort and techniques.

METHODS

This research used exisiting data which are: (1) direct observation of methane emission in Lampung Province from Nugroho*et al.* (1994),and (2) data of paddy field harvest in Lampung from Indonesia Statistical Bureau (2012).

Estimation of annual methane emission.

Methane emissionfrom paddy field was calculated based on mathematical model released by IPCC(2006). This model has been applied by some researchers such as Gupta *et al.* (2008) and BPPT (2009). In this research different ecosystem of the paddy field was ignored since the observation data came from an experiment in one paddy field area, so it is assumed having the same ecosystem.

$$CH_4 Rice = \sum_{i,j,k} \left(EF_{i,j,k} x t_{i,j,k} x A_{i,j,k} x 10^{-6} \right) \quad (1)$$

CH_4 Rice = annual methane emission from paddy field area ($GgCH_4$/year).

EF*ijk* = daily emission factors for i, j, and*k*conditions($kgCH_4$/ha/day).

t*ijk* = paddy time periodfor i, j, and k conditions (day).

A*ijk* = annual paddy harvest area for i, j, and k conditions(ha/year).

i, *j*, and*k* = represent different ecosystem, water rezym, changes in type and amount of organic matter and other paddy field condition which is possible to have different CH_4 emission.

Forecasting annual emission.

Paddy field area in Lampung was applied on mathematical model in order to get annual methane emission in Lampung Province. Data from the estimation would be used as databaseto forecast methane emission for next 5 years period using Box-Jekinsmethod (ARIMA model).

Box-Jekinsmethod (ARIMA model) was developed through identification and estimation steps (Makridakis, 1999). In identification, model is tentatively categorized; from

this stage data could be identified whether it is random, stationer or seasonal, and whether there is AR (*auto regressive*), MA (*moving average*), or both ARMA(*auto regressive moving average*)proceses. Next step is estimating parameters of the tentative model. This step includes non linier estimation, parameter test and model fitness. With those approaching the best ARIMA model for the forecasing would be achieved.

Eventually, this research will be come up with a graph that shows the tendency of the methanee emission from paddy field area in Lampung Province in the near future (up to 2017).

RESULTS AND DISCUSSIONS

Direct observation of methane emission from Paddy field had been done in Lampung (Nugroho *et al*., 1994a and 1994b), the result is presented in Table 1.

Table 1.Methane emission from paddy field in Lampung Province.

Season	Month	Week	Emission (mg $CH_4m^{-2}h^{-1}$)
Rain (wet)	December	4	10
	January	1	18
		2	25
		3	22
		4	25
	February	1	20
		2	25
		3	19
		4	18
	March	1	9
		2	7
Dry Season	May	1	15
		2	30
		3	18
		4	24
	June	1	40
		2	42
		3	20
		4	27
	July	1	17
		2	19

The result of applying equation 1 using data from direct observation of methane emission and data of paddy harvest area is presented in Table 2 and Figure1.

Table2.Paddy harvest area and estimated methane emission in Lampung Province, Indonesia.

Year	Area (ha)	Methane emissions Gg/year	Year	Area (ha)	Methane emissions Gg/year
1993	433,078	163,703	2003	472,635	178,656
1994	425,940	161,005	2004	495,519	187,306
1995	514,363	194,429	2005	496,538	187,691
1996	515,192	171,645	2006	494,102	186,771
1997	454,087	197,155	2007	524,955	198,433
1998	521,575	180,268	2008	506,547	191,475
1999	476,899	187,820	2009	570,417	215,618
2000	496,879	189,423	2010	590,608	223,250
2001	501,119	179,724	2011	606,973	229.436
2002	475,461	178,656	2012	626,158	236,688

Source of paddy harvest area: Badan Pusat Statistik(2012)

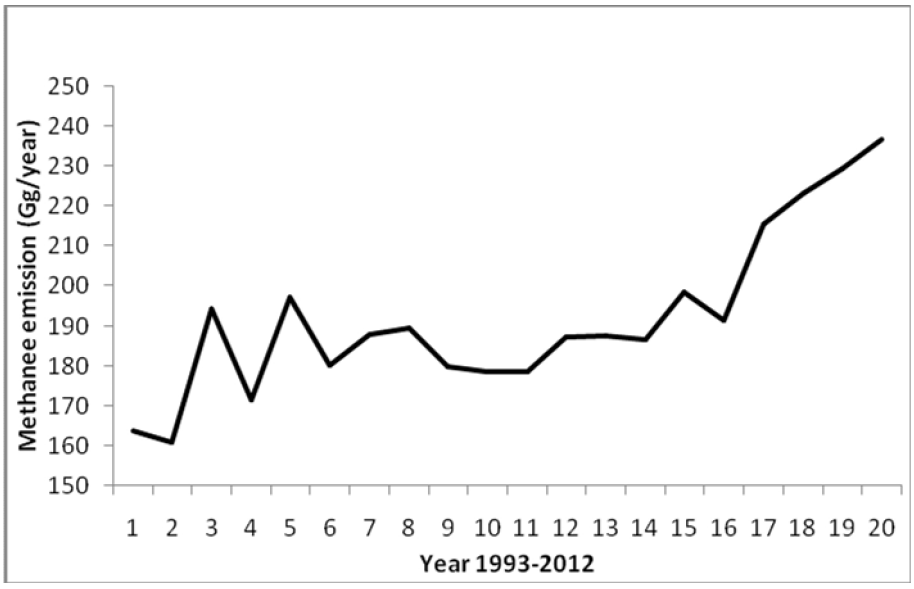
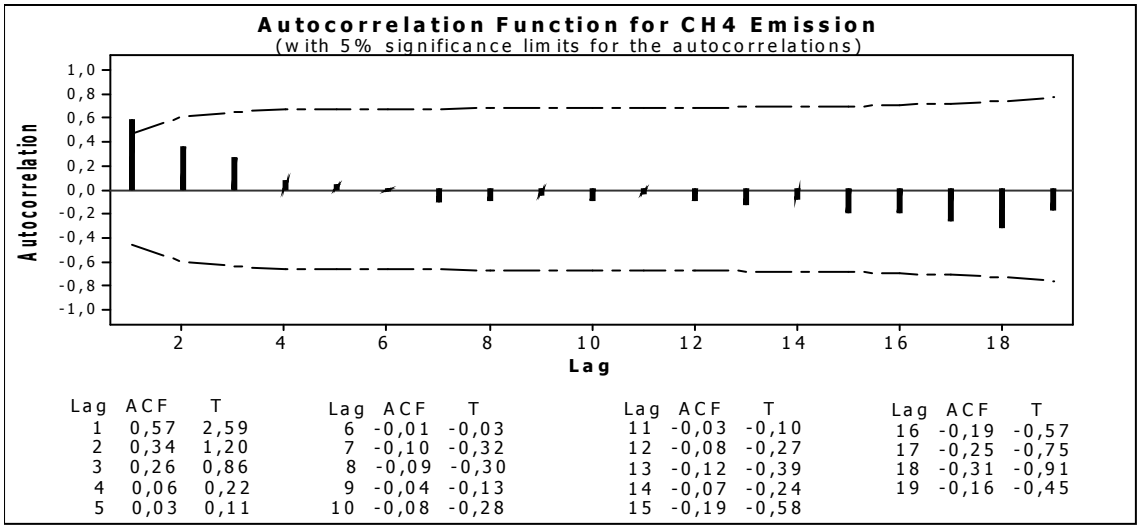


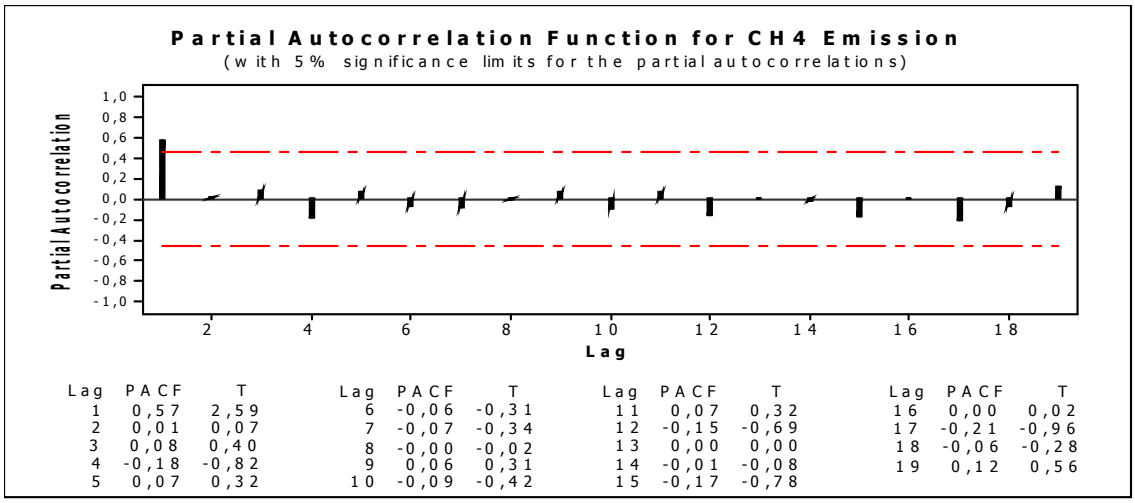
Figure 1.Estimated methanee emission from paddy field area in Lampung Province, Indonesia (1993-2012)

Next step is to calculate autocorrelation and partial autocorrelation; the resuts are presented in Figure 2 and 3.



Lag	ACF	T	Lag	ACF	T	Lag	ACF	T	Lag	ACF	T
1	0,57	2,59	6	-0,01	-0,03	11	-0,03	-0,10	16	-0,19	-0,57
2	0,34	1,20	7	-0,10	-0,32	12	-0,08	-0,27	17	-0,25	-0,75
3	0,26	0,86	8	-0,09	-0,30	13	-0,12	-0,39	18	-0,31	-0,91
4	0,06	0,22	9	-0,04	-0,13	14	-0,07	-0,24	19	-0,16	-0,45
5	0,03	0,11	10	-0,08	-0,28	15	-0,19	-0,58			

Figure2. Autocorrelation function (ACF) of estimated methane emission from paddy field Lampung Province from 1993 to 2012.



Lag	PACF	T	Lag	PACF	T	Lag	PACF	T	Lag	PACF	T
1	0,57	2,59	6	-0,06	-0,31	11	0,07	0,32	16	0,00	0,02
2	0,01	0,07	7	-0,07	-0,34	12	-0,15	-0,69	17	-0,21	-0,96
3	0,08	0,40	8	-0,00	-0,02	13	0,00	0,00	18	-0,06	-0,28
4	-0,18	-0,82	9	0,06	0,31	14	-0,01	-0,08	19	0,12	0,56
5	0,07	0,32	10	-0,09	-0,42	15	-0,17	-0,78			

Figure 3.Partial autocorrelation function (PACF) of estimated methane emission from paddy field Lampung Province from 1993 to 2012.

Both graphs could be indicators whether the data is random, stationer or seasonal, or having AR, MA, or ARMA processes. The stricked dotted line shows the upper and lower border of the coefficient values.

Determination of random pattern

The data is considered random when the coeficient value is inside the borders. The ACF, showsr_1 = 0,579 bigger than 0,438 (the upper border). It means autocorrelation coefficient when k = 1 significantly different from zero. When k> 1, allautocorrelation coefficients do

not significantly different from zero. The same result is showed by the PACF, when$k$ = 1,r = 0,579 bigger than 0,438. It means autocorrelation coefficient when k = 1 significantly different from zero. When k> 1, allautocorrelation coefficients do not significantly different from zero. Therefore, it can be concluded that the data series are random.

Determination of Stationary

The ACF did not show a diagonal trend from left to right as the time lags increases (Figure 2); this proved that the data is stationer; therefore, no data differentiation is necessary. Stationer data has constant mean and varian, there is no up and down pattern. With this result prediction of methane emission the ordo is 0 (d = 0) since no data differentiation is needed.

Determination of Seasonal Trend

The autocorrelation on the ACF did not show a repetition; it means on the ACF no identification that the coefficient on two or three time lags significantly different from zero; therefore, it can be concluded that no seasonal influence on the data series.

Identification of AR (*autoregressive*) processes

The ACF shows autocorrelation values which decreases exponentially (r_1 = 0,579 >r_2 = 0,346 >r_3 = 0,266 >r_4 = 0,069 >r_5 = 0,036), until reach zero after 2 and 3 time lags; that shows the existence of AR proceses. The ordo of AR processes could be determined from the numbers of partial coefficients in PACF that is significantly different from zero, in this study the ordo is one p= 1. The existence of AR process shows that the last data has a correlation with the previous data series and the correlation decreases with further time lags.

Identification of MA (*moving average*) processes

MA processes could be identified from the value of partial autocerrelation in PACF that decreases exponentially. Since there is no indicator of that pattern in this data series; it can be concluded that MA processes did not exist, or the ordo is zero (q = 0). Moving average exists when data has connection with previous data in short time (having short memory)

From those identification steps eventually the ARIMA model which is tentatively suitable for the data series is ARIMA (1,0,0) model. However to determine the ordo for that model, besides the identification steps, it is also necessary to do the “trial and error” steps to obtain ordo comparisons in order to achieve better model. In this study ARIMA (0,0,1) model

is chosen as the alternative model, then parameter estimation would be done for those two tentative models. The model analysis for ARIMA (0,0,1) and ARIMA (1,0,0) are presented in Table 3.

Table3.Statistic analysis model ARIMA (0,0,1) and ARIMA (1,0,0)

Type	Coef	SE Coef	T	p	MSE
MA 1	-0,6985	0,1671	-4,18	0,001	267.68
AR 1	0,9012	0,1773	5,08	0,000	195,91

For α = 0,05; |*t*|value for MA(1) parameter (4,18)washigher than $t_{0,025(24)} = 2,064$. This shows that parameter estimation value of those models were significantly different from zero (reject H_0). Value of *p* parameter of MA (1) was 0,001; much lower than significant level 0,05; means reject H_0. Therefore, the MA (1) model could be accepted.

For α = 0,05; |*t*|value for AR(1) parameter (5,08)was higher than $t_{0,025(24)} = 2,064$. This shows that parameter estimation value of this model were significantly different from zero (reject H_0). Value of *p* parameter of AR (1) was 0,000; much lower than significant level 0,05; means reject H_0. Therefore, the AR (1) model could also be accepted.

Both of the models could be used for methane emission forecasting, the results are shown in Figure 4 and 5.

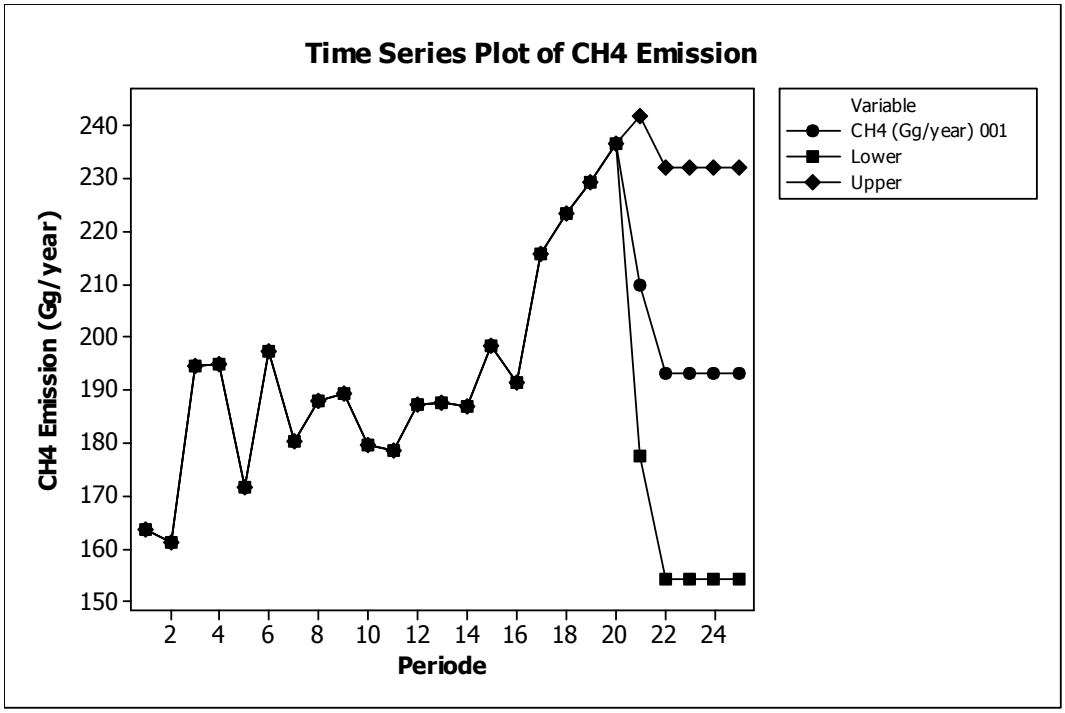


Figure 4.Forecasting of methanee emission using ARIMA (0,0,1) model.

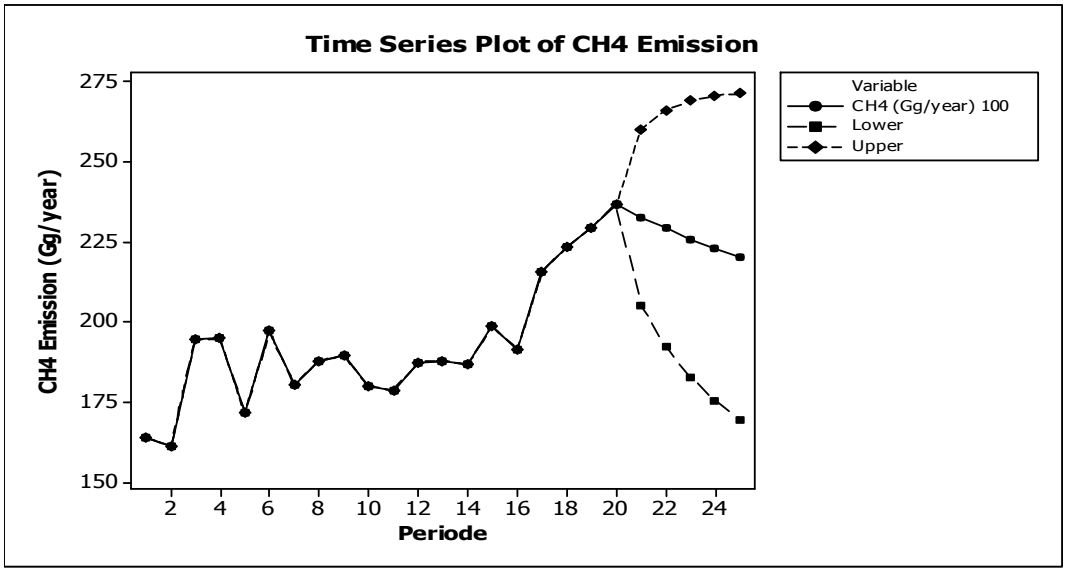


Figure 5.Forecasting methanee emission using ARIMA (1,0,0) model.

From those models, the most suitable model should be chosen. The criterias for chosing the model are: (1) should have less means square error (MSE) and (2) should have more simple equation (see Table 4).

Table4.Level of MSE and model equations

Model	MSE (mean square error)	Equation
ARIMA (0,0,1)	267,68	$X_t = \mu + e_t - \theta_1 e_{t-1}$
ARIMA (1,0,0)	195,91	$X_t = \mu + \Phi_1 X_{t-1} + e_t$

Based on those criteriasARIMA (1,0,0)model was chosen to forecast methane emission from paddy harvest area in Lampung Province in near 5 years future (Figure 5 Table 5).In general from the data series can be concluded that paddy harvest area in Lampung Province is stationer, non seasonal and has strong correlation with the previous area.

Table 5.Forecasting of methane emission in 5 years near future based on ARIMA (1,0,0) model.

Period	Methane emission	Lower border	Upper border
21	232,703	205,264	260,143
22	229,113	192,175	266,050
23	225,877	182,739	269,015
24	222,961	175,378	270,544
25	220,333	169,425	271,241

The tabel shows the forecasting in 5 years of methane emission range from paddy harvest area in Lampung Province.The upper border might be reached if the harvest area and the planting intensity increases which is possible since Lampung province is one potential center of rice paddy production in Indonesia.Research of methane emission from paddy field in other province in Java Island, Indonesia from 2006—2008had been done (BPPT, 2009).

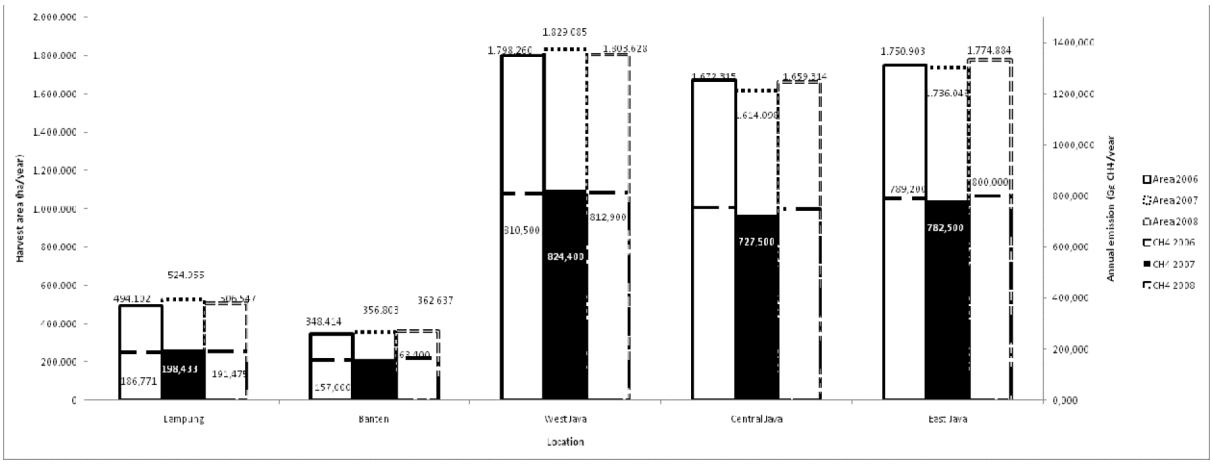


Figure 6.Methane emission from paddy field in provinces of Java Island, Indonesia (BPPT,2009).

Comparing the ratio of the area and methane emission in those provinces shows that in general the ratio was 1:2.000.In Lampung in 2007 the methane emission was 198,433 Ggand the area was 524.955 ha (1:2.645), while in Banten the emission was 160,800 Ggwith harvest area 356.803 ha (1:2.219). From the ratio, it can be concluded that the model in this study could be applied in forecasting methane emission from paddy field.

Comparing the results on this study with similar study conducted in Taiwan (Yang and Chang ,2001) is shown in Figure 7. In Lampung, the methane emission was about 0,161—0,236 Tg/year withpaddy field area was about 425.940—626.158 ha; while in Taiwan the emission was 0,032—0,062 Tg/year with the paddy area was 182.807—277.498 ha.

Similar results has also found in a research in some locations in India (Gupta *et al.*, 2008) see Figure 8.

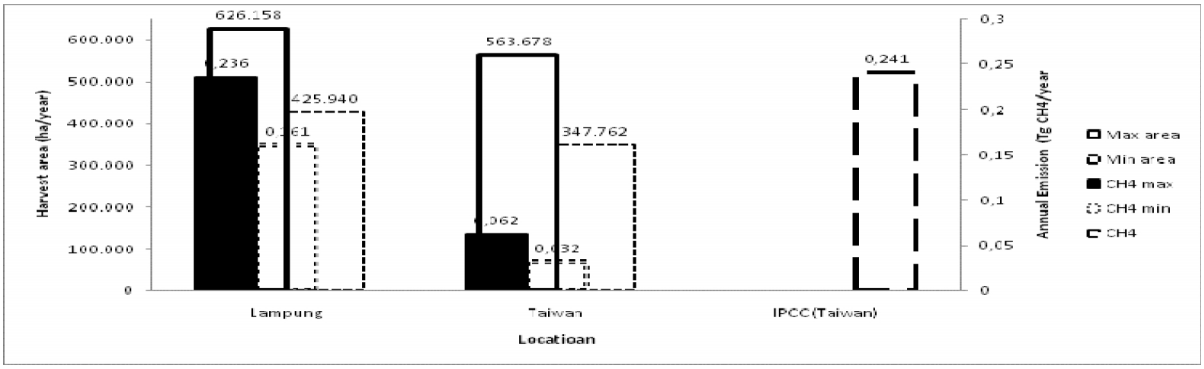


Figure 7.Comparison of methane emission from paddy filed area in Lampung and Taiwan.

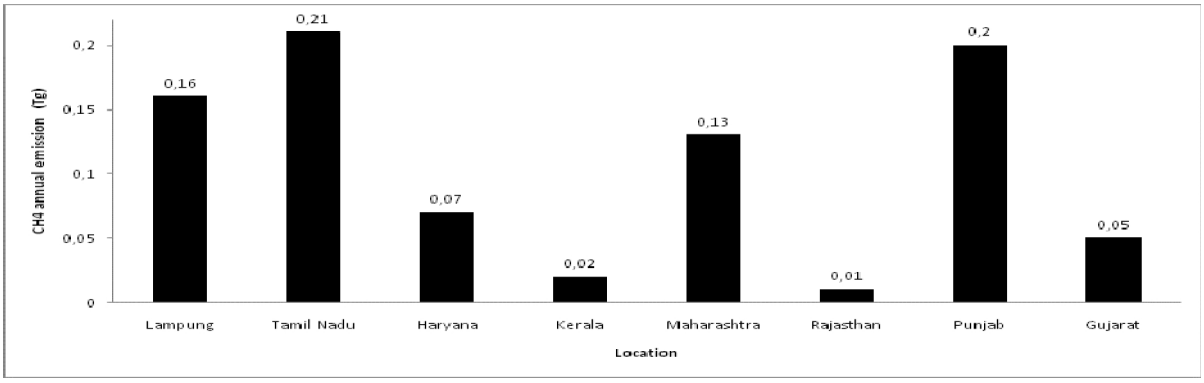


Figure 8. Comparison of methane emission of Lampung and some areas in India

This study showed the possibility of decreasing methane emission in Lampung Province due to decreasing paddy field area. Decreasing paddy field area could happen because of exchanging land use. Some factors influence exchanging land use in farmers level are (1) 97,5% because of no irrigation facilities, (2) 92,5% because the prize of other substitute commodities are higher, (3) 43,4%because of low rice prize, (4) 52,5% because planting paddy does not economically beneficial , (5) 32,5% because of labor scarcity (BPTP Lampung, 2011).

However, even with decreasing possibility of methane emission in Lampung Province, the emission itself is considered high. With long life stay in the atmosphere, increasing rate of methanee emission will significantly effect the climate change processes.

Based on data from Indonesian Statistic Bureau (BPS, 2012) paddy field area in Lampung Province does not always increase. In general, average increasing rate of paddy field area in Lampung Province from 2001 to 2009 was 1,57% per year or about 2.626 ha per year (BPTP Lampung, 2011). On national scale, in 2010 Indonesian government budgeted to develop 62.000 Ha new paddy field and 100.000 Ha in 2012 and plan to develop 100. 000 Ha every year (Dirjen Perluasan lahan, 2013).Badan Pusat Statistik (BPS, Indonesia Statistic

Bureau) predicts rice production in 2015 will increase 6,64 percentor 75,55 million tonscompares to the previous year; and this will be the highest in last 10 years; while the need is only 28 million tons.

Even though the need for rice is decreasing, rice will still be Indonesian staple food. Therefore, it is necessary to apply some mitigation techniques in paddy cultivation. Some of the possible cultivation techniques are: using paddy varieties with low emission, shorter growth time, drought tolerant,moderate fertilizer application, and manage the paddy field water regime.

This study has some limitations because data of direct methaneemission was observed once a week; it should be better if daily observation was available. However, the IPCC model was a common model used in many countries and the ARIMA model is also broadly used in forecasting future data based on previous time series data.

Applying IPCC mathematical model, methane emission from paddy planting area in Lampung Province, Indonesia on the period of 2013—2014were232,703 Gg (2013); 229,113 Gg (2014); and with applying ARIMA model the emission were predicted as 225,877 Gg (2015); 222,961 Gg (2016); and 220,333 Gg (2017).The combination of these models could describe in general the amount of regional methane emision with the assumptions that agriculture managements in paddy cultivation such as water regimes and fertilizer applications are remain the same.

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FARM PERFORMANCE AND PROBLEM AREA OF COCOA PLANTATION IN LAMPUNG PROVINCE, INDONESIA

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ABSTRACT

In the last ten years, the trend of cocoa field productivity in this province was decreasing. This study aimed to explore farm performance and problems of cocoa plantation in Lampung. Two regencies representing low land and mountainous land of cocoa smallholder plantations were purposively chosen; in which 500 farmers were randomly drawn to be interviewed from two districts of every regency with large cocoa areas. Field survey was conducted at one cocoa village with highest elevation of black pod disease. We found that cocoa farming system in Lampung was mixed cropping 60.6% and monoculture 39.4%. Coconut and banana were the main mixed crops with importance value (IV) 94.3% and 37.4% and yield index 134% and 119% respectively. Meanwhile, *Parkiaspeciosa*, rubber, clove, coffee, durio, nutmeg, and long pepperwere minor mixed crops. A 94% of sample farmers figured the fall of cocoa yield was because of black pod disease. Irregular pruning and less farm sanitation especially on waste of pod husk and disease infected pod mayinduced black pod disease. From 9 national clones we calculated the severity score was 2.79 (0-10 score level) and from 4 local clones the scoreswas 4.46. However, Sul2 as one of national clones indicated as a tolerant clone with the lowest score of 0.91.

Key words: cocoa, clone, disease, mixed crops, severity, tolerant, yield index

INTRODUCTION

Indonesia is the world's third largest producers of cocoa. Its production in 2013/2014 was 375 thousand tonnes, estimated to drop to 300 thousand tonnesin 2015/2016 (ICCO, 2016). The plantation area in 2015 was 1.6 million hectars of small holders and 39 thousand hectares of private plantations (Directorate General of Estated Crops, 2014), including 68 thousand ha in Lampung Province.

In 2002-2014 cocoa plantation area in Lampung increased by 11.1% but production increased slightly by 7.8% per year. Figure 1 shows the gap between cocoa planted area and production becomes wider indicating the decreasing of productivity. Table 1 exhibits that the productivity of total planted land (P/A) decreased from around 0.6 to 0.4 ton/ha of cocoa bean and the productivity of mature cocoa land (P/M)decreased from 1,0 to below 0.9 ton/ha in the last ten years.Index of productive cocoa land (M/T) also reduced.Productivity and production of cocoa in this province tendto decrease. This study aimed to explore farm performance and problems of cocoa plantations in Lampung.

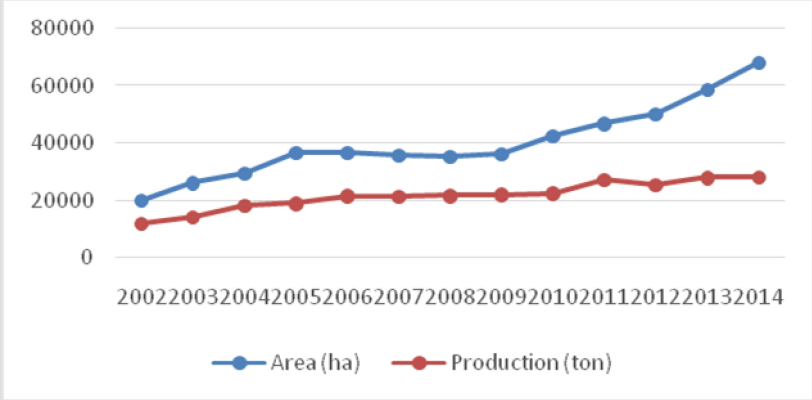


Figure 1. Trend of planted area and production of cocoa bean in Lampung

Table 1. Figures of Lampung cocoa plantation in 2002-2014

Year	Young (Y, ha)	Mature (M, ha)	Old (ha)	Y/M	Total area (T, ha)	M/T	Production (P, ton)	P/T	P/M
2002	7,549	12,005	561	0.63	20,115	0.60	11,579	0.57	0.96
2006	14,263	21,379	966	0.67	36,597	0.58	21,548	0.59	1.00
2008	11,674	22,780	1,003	0.51	35,457	0.64	21,662	0.61	0.95
2010	17,962	27,454	1,042	0.65	46,458	0.59	26,598	0.57	0.97
2011	19,441	29,451	1,051	0.66	49,943	0.56	25,541	0.51	0.87
2012	22,823	29,994	1,015	0.76	53,832	0.56	28,495	0.53	0.95
2013	27,287	33,532	1,436	0.81	62,265	0.54	30,907	0.50	0.92
2014	35,014	32,057	1,081	1.09	68.152	0.47	28,067	0.41	0.87

Note: Young = not yet productive, mature = productive, Old = unproductive

Source: Calculated from Statistics of Lampung Province, 2003-2015

METHODS

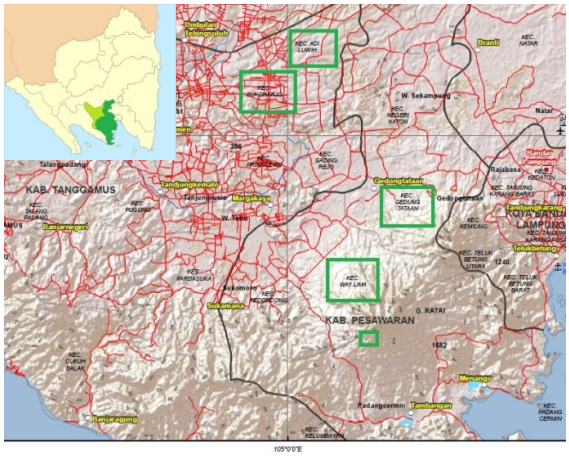
Field Study

The site of this survey covered two regencies of Lampung Province which were purposively sampled representing low land (Pringsewu Regency) and mountainous land

(Pesawaran Regency) of cocoa smallholder plantations. In each regency we purposively sampled two districts with large area of cocoa, and randomly sampled 500 farmers to be interviewed. The sampled districs were Sukoharjo and Adiluwih of Pringsewu and Gedung Tataan and Way Lima of Pesawaran Regency. We askedabout cocoa land area, cocoa age, cocoa population, cocoa plant management, pests, diseases, yield and revenue from cocoa and mixed crops.

Field survey was conducted in one cocoa village (Harapan Jaya Village, Pesawaran Regency) with the highest elevation to survey problem of black pod disease in the farms where chemical fungicide were not applied.Fields were purposively sampled to observed severity of national and local clones from Phytophthora pod rot or black pod disease. Cocoa trees having pod disease symptoms were randomly sampled.

Pringsewu Regency is located at 104º42' - 105º08' E and 05º20' - 05º57' S with elevation of 100-200 m above sea level. Pesawaran Regency is located at 104º94' - 105º20' E and 05º14' - 05º77' S with elevation 100-400 m above sea level. Harapan Jaya village is located at 105º05' E and 05º33' S with elevation 400-600 m above sea level. Rainfall is 1500-2300 mm year^{-1} with 2-5 dry months occurring in June – October.



Source: Geospasial PNBP, 2010

Figure 2. Location of study site (in green mark)

Data analysis

We based cocoa trees spacing of 3x3 m with population 1111 trees/ha (Koko et al., 2013) and calculated the effective land for cocoa as percentage of existing population/1111. Analyses of Impotance Value (IV) was modified from Mardi et al. (2014) and Ofori-Bah

&Asafu-Adjye (2011) using frequency and revenueshare (mixed crop revenue/cocoa revenue). It was based on consideration that the bigger the frequency and revenue share, the more important the mixed crops are. We calculate IV as sum of Relative frequency and Relative revenue. We expressed Economic Yield Index (EYI) ofmixed cocoa system as EYI = (1 + mixed crop revenue/cocoa revenue) x 100%.

Based on growth stage of fruit using BBHC scale (Niemenak *et al*., 2010), the rotten fruits were scored. The healthy cherelles, cherelle wilts, healthy and diseased pods (minimum 0,5 cm^2 of black spot) were counted. The scores were 1 for cherelle wilt (BBHC 70-74), 3 for small pods (BBHC 75-76), 5 for big pods (BBHC 77-80), and 1 for ripe pods (BBHC 81-89). Severity score is total of each fruit stages score.

Severity score = ∑ fruit stages score

Fruit stage score = number of disease fruit x score/number of fruits

RESULTS AND DISCUSSION

Cocoa plantations in Lampung have less ideal of plant age structure (Figure 3)in which young productive trees (age of 5-9 year) are dominant (46%) and trees in highest productive phase (10-14 year) are the second (28%).It is supported by data in the last ten years, that the areas of young cocoa rise consistanly by 28% a year, indeces of expansion area (Y/M) shows a positive trend (Table 1). Cocoa plantations in Lampung are smallholders of ≤2 ha land area; mostly (57%) farmers have less than 1 ha (Figure 4). Lampung was a beginning destination of transmigration from Java Island. Starting in period of 1905 – 1922 inhabitants came to along Gedung Tataan (Pesawaran) to Wonosobo (West Kota Agung, Tanggamus) (Levang and Sevin, 1990) that occupied limited land for agriculture (Kingstone, 1990) including for cultivating rice, other food crops, fruits and cash crops such as cocoa, coffee, coconut, and banana.

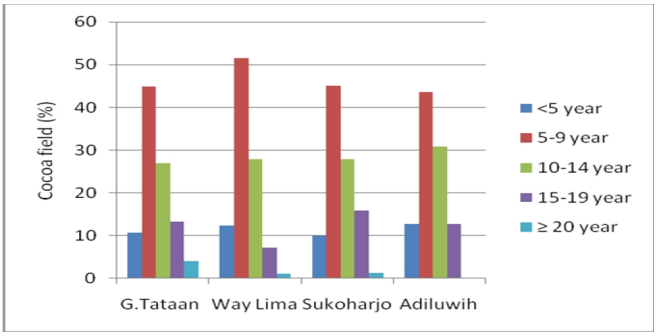


Figure 3. Structure of plant age

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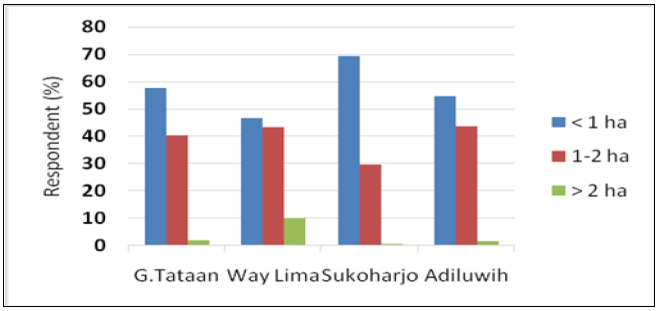

Figure 4. Farmers' cocoa land area

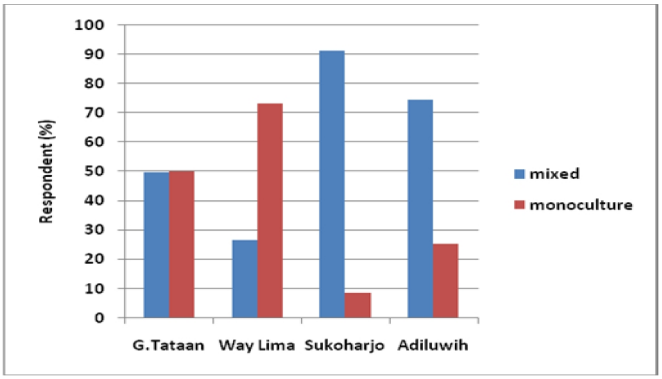

Figure 5. Cocoa fields of mixed culture and monoculture

Most cocoa plantation in Lampung is mixed culture (61%) particularly in ex-transmigration Regencies (Gedung Tataan, Sukaharjo, and Adiluwih) and the rest (39%) is monoculture (Figure 5). Cocoa farmers owning small land tend to cultivate mix crops in the same land to increase land equivalent ratio (LER) and income. Tree diversity within the cocoaproduction systems is variable, depending on management, cultural differences, locationand farm history (Schroth and Harvey, 2007). We found at least 17 tree cropspecies mixed with cocoa including food crops, fruits, spices, timber, and other cash crops that generate family income. The mixed crops were more diverse in Pesawaran District than those in Pringsewu District. Coconut is most common crop found as mixed crop in cocoa field (f=152) especially in Pringsewu District as a lowland, followed by banana (f=69). Based on importance value (IV), the important mixed crops found in cocoa field were coconut, banana, Parkiaspeciosa, and rubber with the value 94.3%, 37.4%, 17.38, and 17.33% respectively (Table 2). Intercropping cocoa-coconut is commonly recommended due to high yield of cocoa bean about 600-2300 kg ha^{-1} (Osei-Bonsu et al., 2002). Cocoa-banana is commonly

usedsince banana gives shade for cocoa trees (Schroth and Harvey, 2007). Parkia speciosa is a legume tree that is commonly found in smallholder plantations in Lampung as its fruit has high economic value and provide shading for cocoa and coffee. Rubber intercropped with cocoa is also recommended as it provides shade, revenue and ecological service (Langenberger *et al*., 2016).

Effective land for cocoa trees (or its population) mixed with coconut, banana, or coffee tend to lower than those with Parkiaspeciosa, durio, rubber tree, or cloves. Mixed cropping with coconut, banana, and coffee reduced population of cocoa trees. Coconut and banana was the most popular mixed crops for cocoa. Coffee is a traditional crop in medium and high altitude land while rubber tree is common in low altitude land. Dupraz and Morisson (2015) stated that cocoa trees could gradually replacing and mixed cropping with traditional crops including coconut, coffee, rubber, and banana that developed various types of diversification.

Table 2. Importance value index of cocoa mixed crops

Mixed crop	Pesawaran District				Pringsewu District				IV aver
	Gedung Tataan		Way Lima		Sukoharjo		Adi Luwih		
	% Rev (F)	IV	%Rev (F)	IV	%Rev (F)	IV	%Rev (F)	IV	
1. Coconut	8.07(8)	11.52	14.23(3)	23.86	33.99(105)	162.77	36.34(36)	179.07	94.30
2. Banana	18.28(35)	68.98	7.80(7)	42.69	18.65(24)	28.26	11.42(3)	9.71	37.41
3. Parkia speciosa	34.07(9)	25.12	36.27(3)	42.95	0(2)	1.45	0(0)	0	17.38
4. Rubber	14.07(18)	31.53	10.18(5)	33.92	0(2)	1.45	0(1)	2.44	17.33
5. Clove	26.82(3)	7.24	29.52(2)	24.73	0				7.99
6. Coffee	55.61(4)	15.64	24.19(1)	10.83	0				6.62
7. Durio	11.68(8)	13.02	2.06(3)	13.32	0		0	0	6.58
8. Nutmug	19.57(8)	16.30	0	0	0				4.07
9. Long pepper					98.18(1)	3.11	90.94(1)	8.78	2.97
10.Kayu Jabon			0(2)	7.69	0				1.92
11.Candle nut	22.96(2)	4.43	0(0)	0	0				1.11
12. Kapuk	30.30(1)	2.59		0	0				0.65
13. Avocado	17.54(1)	1.93		0	0				0.48
14.Salak	12.50(1)	1.67	0		0				0.42
15.Waru					0(2)	1.45			0.36
16. Pinang					2.29(1)	0.78			0.19
17. Acasia sp.					0(1)	0.72			0.18
Sum	(n=98)	200	(n=26)	200	(n=138)	200	(n=41)	200	

Mixed cropping cocoa with other trees generated more revenues. Economic yield index of mixed cocoa system was about 110-135%, but it tend to be higher in cocoa-coconut, cocoa – P. speciosa, and cocoa-coffee (134-135%) than othercropping systems. Bean yield of cocoa mixed with various cash treeswas about 790-860 kg/ha/year. Itsvariabilitywas high with

standard deviation about 150-520 kg.Compared to other reports, this yield was considerably low. Mixed cropping cocoa-orange or cocoa-avocado resulted about 1.3 ton ha^{-1} $year^{-1}$ cocoa bean (Koko *et al*., 2013). Associating timber trees with cocoa did not negatively affect cocoa yield (Somarriba and Beer, 2011). Diversified (or mixed) cocoa farms are more efficient than single crop (or mono-crop)cocoa farms, indicating possibilities for cost complementarities between production of cocoa and mixed crops (Ofori-Bah and Asafu-Adjye, 2011).

Productivity of cocoa field on average in 2011-2013 is presented in Figure 6. In 2011-2012 productivity ranged from 1300 to 1500 kg ha^{-1}. In 2013 productivity dropped to about 800 kg ha^{-1}. During the focus group discussions it revealed that in 2014 the productivity drops by nearly 50% so that some farmers converted cocoa plants to rubber or nutmeg plants. The decline in productivity occured due to pests (particularly *Conopomorpha cramerella*) and diseases (particularly black pod) as well as low rainfall followed by high rainfall. According to Wood and Lass (2001), annual rainfall in excess of 2500 mm may lead to a higher incidence of fungus diseases, particularly *Phytophthora* pod rot (black pod disease). Moreover, Adjaloo et al. (2012) reported that rainfall positively affected flowering and phonological cycle.In addition, Sabatier et al. (2013) concluded that the behavior of cocoa agroecosystems (pests, diseases and yields) depends on its management.

Table 3. Effective land for cocoa, economic yield index, and cocoa bean yield

Mixed crops in cocoa field	Effective land for cocoa[1] (%)	Cocoa bean yield (kg/ha)	Economic yield index (%)
Coconut	73.51± 24	792.16 ± 519	134.10 ± 21
Banana	73.71± 16	863.51 ± 469	118.93 ± 22
Parkia speciosa	81.13± 19	801.42 ± 381	134.61 ± 29
Durio	97.61± 38	816.60 ± 361	110.15 ± 12
Rubber	80.26± 28	845.10 ± 455	116.18 ± 21
Clove	80.41± 12	855.74 ± 149	120.96 ± 20
Coffee	72.13± 15	854.19 ± 300	135.51 ± 13

Note: 1. based on spacing 3x3 m, population 1111

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Since 2011-2013 productivity declined. In 2013, in Gedung Tataan and Way Lima, productivity was dominated (82%) by low (<500 kg/ha) and middle levels (500-1000 kg/ha). In Sukoharja the productivity are quite similar among the levels. In Adi Luwih, productivity was dominated by middle and high levels (>1000 kg/ha) (Figure6). Farmers in Adi Luwih showed an intensive maintenance of cocoa farming in terms of pruning, fertilizing, manuring and applying pesticide.In small land tenure of cocoa farming, it is important to achieve high production.

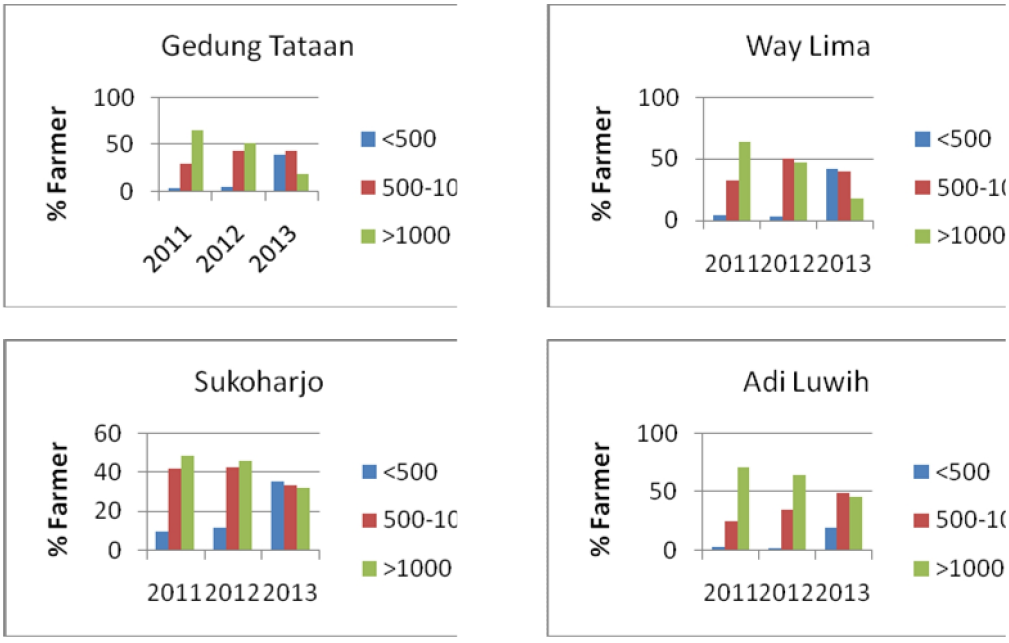

Figure 6. Dynamic of cocoa productivity by district

To achieve high production, farmer in Sukoharjo and Adi Luwih were more eager to expand cocoa plantation. Farmers were optimistic that cocoa farming remains profitable despite some obstacles such as the incidence of pests and diseases and climate change that

cause decreased production of cocoa. For example, black pod disease can be overcome by pruning, sanitation, and planting disease-resistant cocoa clones. Related to climate change, Schroth *et al.* (2016) concluded that adaptation measures are needed such as by selecting cocoa varieties and companion trees and crops that are tolerant to high temperatures, drought and diseases, increasing shade to protect cocoa trees and diversifying farmers' income as a buffer against market and environmental risks.

Table 4 showed the important cocoa diseases in Lampung. The most important disease is black pod disease by *Phytophthora palmivora* causing significant lost of cocoa yield in the last 3 years in Lampung. Another important disease is trunk cancer also caused by *Phytophthora palmivora*. Climate change, applying too much fungicide, improper pruning, and bad sanitation may induce the fungi to spread. Another disease called vascular steak dieback (VSD) caused by *Oncobasidium theobromae* is not much important in Lampung.Controlingcocoa plant disease (including black pod) could be achieved if an integrated management strategy is established, with the combination methods such as phytosanitaryand other cultural measures, and selection for resistant clones (Acebo-Guerrero et al., 2012) including local clones from open-pollinated progenies (Thevenin et al., 2012).

Table 4. Types of diseases that attack cocoatrees in Lampung

Diseases	Pesawaran (of 100% respondent)		Pringsewu (of 100% respondent)	
	Gedung Tataan	Way Lima	Sukoharjo	Adi Luwih
Black pod	90.2	90.0	97.4	98.2
VSD	4.9	6.7	91.4	92.7
Trunk cancer	45.1	44.4	64.9	76.4

In average, 66% of respondents did branch pruning irregularly, so that the cocoa trees were 3-4 high, much branching, dense canopy, low light interception, and high air humidity. It makes cocoa trees susceptible to black pod disease. Black pods were remaining hang on the tree that becomes the source of infection. Moreover, farmers harvest ripe pods and commonly peel it at the field (70% respondent) and left it on the ground without any treatment (72% respondent). Only 10% of farmers used cocoa peel as fodder, commonly for goat (Table 5).

Table 5. Branch pruning, pod peeling, pod waste management

Activities	Treatment	Pesawaran		Pringsewu		Average
		Gedung Tataan	Way Lima	Suko-harjo	Adi Luwih	
Pruning	1 x / year	4.4	5.6	17.9	10.9	9.7
	2 x / year	19.6	10.0	43.7	43.6	29.2
	3 x / year	1.5	0.0	4.0	5.5	2.7
	Irregular	74.5	84.4	34.4	40.0	66.3
Place to peel pod	Field	93.1	97.8	21.2	18.2	69.7
	Home	6.4	2.2	78.1	78.2	28.9
	Field and home	0.5	0	0.7	3.6	1.4
Pod waste management	No treatment	76.0	94.4	57.6	58.2	71.5
	Burning	0	0	3.9	1.8	1.4
	Composting	8.8	2.2	17.2	7.3	8.9
	Buried	0	0	12.6	20	8.1
	Animal fodder	15.2	3.3	8.6	12.7	9.9

This cocoa tree management practices resulted in high incident of black pod especially when season of rainy and foggydays, and the cocoa bean yield has falled below 500 kg/ha/year since year 2013.Moreover, most farmers (77%) cultivated local cocoa cultivars which are susceptable to black pod disease. Farmers made seedlings by themselve using local seed resource. So far, neither local clones nor national clones were really resistant to black pod disease. We observed 4 selected local cloneswhere50% of trees had incidence of the disiease with severity scores 4.46. From 9 national clones we calculated the severity score was 2.79. However, Sul2 of national clone was indicated as a tolerant clone with the lowest score of 0.91 followed by RCC 70 and TSH 858 with scores of 1.49 and 1.83 respectivel (Table 6). Theseclones had low severity score because incidents of black pod were found at cherelle, small, or ripe pods, while their big pods were free from black pods (Figure 7). MCC1 (well known as M 01 clone) selected for high yielding clone was a moderat-tolerant to black pod disease (McMahon et al., 2015).Gradient resistance of black pod disease may occure in testing of genotypes of cocoa (dos Santos *et al.,* 2011).

Table 6. Severity score of black pod disease of national and local clones

	Clone	Diseased Tree (%)	Severity Score
National	RCC 70	36.15	1.49
	RCC 71	21.05	5.83
	MCC1	34.62	2.39
	Sul 3	26.09	2.55
	ICCRI 7	23.08	2.57
	Sul2	21.42	0.91
	ICCRI 3	18.18	2.77
	TSH 858	23.81	1.83
	Sul 1	34.61	4.77
Average		**27.67**	**2.79**
Local	LT1	57.14	4.28
	LT7	46.15	5.17
	LP1	54.54	4.98
	LP6	42.86	3.43
Average		50.17	4.46

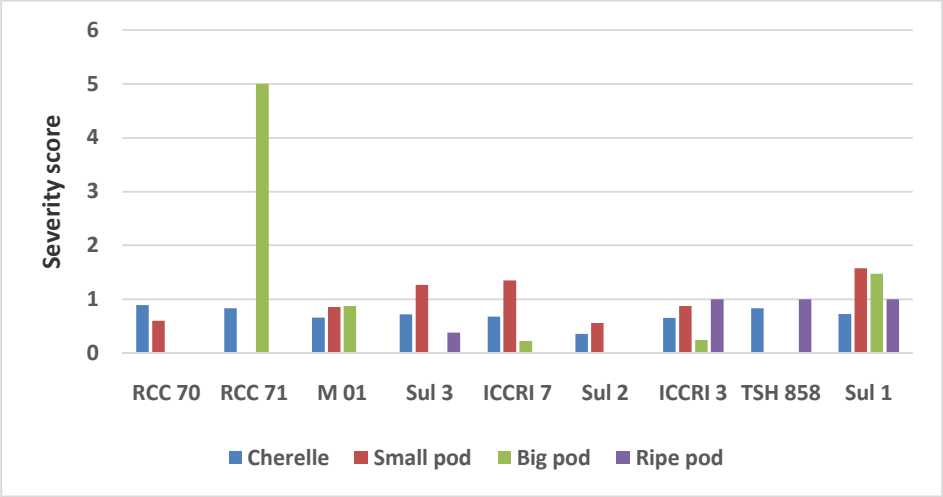

Figure 7. severity score breakdown in fruit development

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NATURAL RESOURCES AND ENVIRONMENTAL MANAGEMENT BY PARTICIPATORY MODEL IN SUPPORTING FOOD SECURITY AND FAMILY INCOME AT DRY LAND FARMING SYSTEM IN SEMAU ISLAND

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ABSTRACT

An appropriate environmental management based on characteristic site is a promising concept to support sustainable agricultural production (food crop, estate crop, forestry, and livestock), family income, family nutritions and food security. This concept is very important to be applied at semiringkai zone which is dominated by dry land. This is mainly due to the semiringkai zone is vulnerable to the changes of environmental components (geo-physic, biology, socio-economy, socio-culture, and healthy people) due to climate changes. Study of natural resources and environmental management in Semau Island showed that the environment components (geo-physic and biology) have being degraded gradually. These conditions affected significantly to agricultural production, family income, family nutrition, and food security. One of the main problems caused environmental degradation is inappropriate natural resources management. Some ways have been practiced in minimizing those problems. However, that has not been going to the main problems in particularly people involvement to identify environmental problems that support on their agricultural practicing and how to overcome those problems by using their knowledge. Hence, integrated participative approach is a promising way to improve degraded environment which affect significantly to the agricultural practices. The concept of integrated participative approach is that interaction between farmers, scientific people (student and lecturer), profesionals, practisioners and NGO to identify the main natural resources and environmental problems and their potential, to determine improvement concepts, and able to be practiced based on their knowledge. Hopefully, by practicing those concepts, the degraded natural resources and environment can be improved and can affect to improvement of soil and plant productivity, family income, family nutrition, and food security

Key words: natural resources, enviromental management, participatory approach, food security, family income, family nutrition, dry land

1. INTRODUCTION

Semau island consisted of two sub districts, they are Sub district of North Semau and Sub district of South Semau, district of Kupang, East Nusa Tenggara Province. The main transportation to link district of Kupang to those sub district of Semau is a boat which able to carry about 20 people and 5 motor cycles. There are four small harbors to be used as landing points for boat which located at Hansisi vilage, Ulasa vilage, Otan vilage and Bokunusan villages. Most land area is dominated by flat to hilly by slope is about 5-15%. Main soil is Litosol which has pH about 6,0 -8,5, limited clay (kaolinit), and hight holding nutrient capacity, hence, availability of some nutrients are limited such as Nitrogen, and Phosphor (Anonimous, 2005). Agriculture farming system is dominated by dry land farming system with low diversity. Most dry farming system are shifting cultivation, moor cultivation, yard cultivation, and traditional forest cultivation. Main crops have be grown are maize, mung bean, ground nut, casava, and paddy field (Badan Pusat Statistik, 2003; Anonimous, 2004 b)
Early studi showed that biodiversity at Semau island tend to decrease gradually. This is mainly due to in appropriate soil management, agricultural practised which is lack of soil conservation, and decreasing of forest capabilitu. All these practised affect to decreasing in soil capability (chemical, physical, and biological). Moreover, *Chromolaena odorata* invation affect significantly to decreasing biodiversity at Semau island. Decreasing biodiversioty might able to cause decreasing in environmental capability which takes along time to recover on early condition or even migh not be recover potentially (Monk, de Fretes, and Reksodiharjo-Lilley, 1997; Soetedjo and Mudita, 2002).

Those low enviromental capability affect significantly to sustainability crops yield grown by farmer which are mostly to fulfillment family needs. Some of crops such as chili, onion, and seaweed are trade to Kupang. However, most crops are trade locally to traditional market aor sell dor to door. Finally, it will cause decrease family income, nutrients value, and food security. Lack of farmer skill to manage their dry land farming system is also a main variable affect to decreasing soil and crop production

Based on environemantal capability problems, nutural resources availability, and lack of farmer skill that relate closely, it should be soonly overcome in short term action, medium term action, and long term action. In practical point of view those actions should be combined with interest of villagers group (farmer's group, women's group, teen's group, profesional's group, trader, broker, small businessman, and local officer goverment). A promising way to

overcome those problems is building research competitive and educational field which involve the academic community (lecturer and college student) , Non Governmental Organization (NGO), and farmers community. The academic community is source of knowledge and technology. There are plenty of applied technology which are able to practised on field by farmer. NGO is source of profesional people who have many field experiences in community empowerment and aplying tecnology. Farmers community is object and subject who relate directly to the change of their agricultural environment By develop field participatory model involving those three componets, natural resources and environment particularly on dry farming system migh be managed properly, soil and plant degradation migh be improved gradually, hence, soil and crop prodution migh be improved (Vivian, 1995; Jayant, Gopal, and Albab, 1996; Mikkelsen, 1999). Finally, sustainability of family nutritions, family income, and food security migh be maintained securely.

2. MATERIALS AND METHODS

Study had been done at Sub District of Semau, District of Kupang in Semau island. Three villages had be sampled as representative of terresterial environment (Uitihana), coastal environment (Akle), and mountanius environment (Uiboa). Primary datas collection were observed directly on the field by using stratified random samples (physical, chemical, and biological of environmental componets). The method thereafter were combined indepth interview method by using Focus Group Discussion and Semi Structural Interview.To improve identified problems and potential natural resources and environmental components, it was developed a field school which involve directly (farmers, college students, lecturer, practisioners, and NGO). Results of field school was presented at workshop at village and sub district level(Field, and Burch, 1988; Jayant, Gopal, and Albab, 1996., Mikkelsen, 1999) .

The main aim of the study was learning together between community, scientific community (represented by Agricultural Faculty of Nusa Cendana University, and Pandu Lestari Foundation), and Agricultural Department to identify, analysis, practise, and develop pratical technology in sustainable management of natural resources and environmental components

2.1 Problem and pontential of natural resources and environment

2.1.1.Problem Analysis

The environmental and natural resources problems had been analysed by using secondary datas and primary datas that were collected by field observation and in depth interview

method (Mikkelsen, 1999). Result of the study showed that some problems had been able to identified. They were: (1) lack of water resources, (2) fire , (3) land degradation, (4) natural resources depletion, and (5) sedimentation.

Commentary of environmental and natural resources problems can be showed at Table 1. All identified problems (Table 1), thereafter, were analysed by interacted problems as because and problems as result to decide an important identifed environmental problems. They would be scored by 1 if there were interaction cause and result, meanwhile they would be scored 0 if there were no interaction cause and result. Result of analysed showed that some problems sholud be observed carefully in managing environmental and natural resurces. The problems were ***land degradation, low land cover (degradation biodeversity), burning forest, improperly management, and lack of land conservation***

Table 1. Environmental and natural resources problems analyses at sub district of semau, district of kupang

Problems aspect	Main problem				
	Land degradation	Fire	Sedimentation	Natural resources depletion	Lack of water resources
Natural cause	High rainfall in short periode time and low land cover	Lightning and wood friction	Highly erosion Low land cover		Short day rainfall Innormal rainfall distribution, and low of soil water holding capacity
Artificial cause	Burning Land clearing Land of land conservation, and burning forests	Burning Land clearing, and weeding	Destruction of mangrove, burning, and illegal logging	Illegal logging, 0ver explotation, improperly management	Illegal logging, burning, and land clearing
Economic and social cause	Shifting cultivation, ranch off, lack of counseling, and lack of coordination	Shifting cultivation, ranch off, poverty, and top down approach	The urge of live, illegal logging, and ranch off	Market requirement, the urge of live, lack of counseling, and lack of coordination	Social conflict, top down approach, improperly management
Direct impact	Decreasing soil capability, sedimentation, low of soil water holding capacity	Low lsnd cover, erosion, decreasing in water infiltration, damage to landscape	Damage to reef and river ecosystems	Declininf reserves of natural resurces, lack of regeneration, and destruction of an environmental balance	Reduced water discharge, limiting basic famer needs, and low sanitation
Indirect impact	Decreasing agricultural productivity, decreasing in farmer income	Declining of soil fertility, domination of fire resistent weed, and increasing of degraded land	Reduced catches of fish, and siltation of river	Decreasing in farmer income, social jealousy	Low of family nutrients, low of crop and livestock yield

Sources: data analysed

2.1.2. Potential Analyis

Potencial of environmental and natural resources at sub district of Semau consisted of five main groups. They were : (1) potential natural resource, (2) potential human resource, (3) potential infrastructure , (4) potential institutions, and (5) potential wisdom

Each group of potential consisted of some potential matter which had been separated in two kinds of potential, which are : (1) potential basic such as agricultural land, forest production, fishery, savana, infrastructure etc, and (2) potential derivatives such as forest products, food crops, estate, fishery, drinking water etc

The result of interaction between potential basic and potential derivatives showed that ***agricultural land, fishery, and transportation infrastruture*** were potential basic of physical enviroment and natural resources which should be developed at sub district of Semau considering others basic potential and should be cultivated wisely, and enviromentally friendly. Some studies should be focused more to develop an appropriate technology, practically, and could be replicated at various areas.

2.2. Problem and potential of agricultural sector

2.2.1 Analysis Problems

2.2.1.1. Land Use Model

An area at sub district of Semau, district of Kupang was 248.66 km^2. Most of land were dominated by shurbs (79.73%), and followedby settlement (7.65%), forest (5.56%), savana (4.73%), moor (1.33%), garden (0.79%), dry paddy field (0.20%). Wide forest area was located at Akle village, however, there was no forest product migh be sold as source of family income. Most of forest products were used as fire wood and fence. Main food crops were maize (951 ha), ground nuts (590 ha), upland rice (149 ha) and other food crops. This result showed that agricultural land at Semau was suitable to be practised for maize and this crops was the main food consumed by farmer aprat of rice and others. Total estate land area was 188.4 ha which was dominated by cashew (148 ha), and thereafter follwed by kapok (20 ha), palm (12 ha), and coconut (8.4 ha). Horticultural production was grown at the area which was closely to water resource such as red onion, chili. These crops were main product to increase family income (Anonimous, 2004 b; Badan Pusat Statistik, 2003).

2.2.1.2 Crops Production

The highest total production of food crops at sub district Semau was upland rice and followed by maize, ground nut, casava, rice paddy, wheat, sweet potato, and mungbean (Anonimous, 2004 b). However, result of the study showed that maize was the main food crop consumption. Meanwhile, the highest horticultural products was red onion, and followed by china cabbage, chilli, garlic, tomato, cabbage, and spinach. Moreover, the highest production of annual crops was water melon, followed by mango, papaya, jackfruit, banana, and soursop. All these annual crops were not cultivated intensively and mostly grown at moor or yard. Main estate crops were cashew and coconut. However, yield of those estate crops tended decrease gradually. It mainly due to farmers did not manage optimaly, they did not clean regularly the garden, poor organic matter application etc. On the reality, those crop were promising estate crops to be developed at Semau physical environment, however, it should be interacted positively to social condition of Helong community (Soetedjo and Mudita, 2002).

2.2.1.3 Livestock Production

There were six types of livestock as an important liveststock for family and for trading. They were cow, buffalo, pig, horse, goat, and chicken. Generally, livestock was managed traditionaly. Semi intensive management system was applied limitly to fattening in small amount. Production of those livestock depends strongly to availability of feed, market demand, and ability of farmer in maintaining the livestock. (Soetedjo and Mudita, 2002)

2.2.1.4 Fishery Production

Potential fishery products at district of Semau was overflow. It had been predicted that potential product of fishery was 26000 t/year or about 35.33 % their potential sustainability. Fish catching was limited on coastal area. It mainly due to most of fisherman did not have big boat which could be sailed to the ocean. More over, they use generally traditional fish catching equipment such as fishing rod, tardional trawl, and several various of gillnet. Secondary data analysed showed that mackarel tuna was the highest fishery product (695.4 t/year) and the lowest fishery product was lobster. However, these product was still lower compared their potential yield. This is mainly due to most fisherman use traditioanl fish catching equipment and also it was not their primary farm ability (Anonimous, 2004 a).

2.2.1.5 Forest Production

Some studies showed that forest degradation occur gradually at sub district of Semau. Therefore, forest management was focused more on improving degraded land by reforestation

such as grown multiprupose trees, controling illegal logging, controling collection of firewood ect. It was seemly a promising consept, however, it was practically fail. This is mainly due to lack of analysis main cause that resulted forest degradation and farmer community did not involve directly to in determining main cause of forest degradation, even though the farmer was subject and object of environmental degradation. Most of forest product were teak, kedondong, tamarind tree etc (Fox, 1977; Soetedjo and Mudita, 2002)

2.2.1.6 Analysis Problem at Agricultural Sector

Result of study showed that there were several problems had been identified in developing agricultural sectors. The problems were : (1) limitation of the availability natural resources and environmental components, (2) lack of technology and management in developing agricultural sectors, (3) dificulties on production infrastructure, (4) limitation of farm credit, (5) limitation policy and program, and (5) limitation marketing of products.

The results of interacted among those problems showed that ***limitation of the availability natural resources such as degraded land, and traditional agricultural practises, lack of technology such as lack of skill of farmers*** were main problems to improve and manage agricultural sectors at district of Semau. The problems resulted directly to decreasing of agricultural yield, decreasing of family income, depend strongly to middleman and goverment package.

2.3 **Analysis Potential**

The result of the study showed that there were numbers of potential migh be developed to improve agricultural sectors. They were : (1) the extent of land that were available to develop agricultural sectors, (2) the extent of territorial water that has not been exploitated maximaly, (3) the high willingness of farmers to improve environmental conditions which support their agricultural practises, and (4) supporting of institution.

The result of interacted analysis among the identified potential of agricultural sectors showed that generally, all potential basic of agricultural sectors at district of Semau migh be developed to improve and increase the yield of agricultural products, improve enviromental components, and improve biological diversity. Finally, those would affect significantly to sustainability of soil dan plant productivity, family income, family nutritions, and food security.

3. RESULTS AND DISCUSSION

3.1 Participatory Consept to Manage Natural Resources and Enviromental in Supporting Food Security

Participatory Rural Apprisal Approach by practising Focus Group Discussion (FGD) that represnting the interests of group, and Semi Structural Interview (SSI) designed to the studi. This process worked gradually from people community group at vilage level, scientific community, and people community at sub district level. Result of the proceses always be discussed at the workshop in village and district level. Main aim of the studi was learning together between community, scientific community (represented by Agricultural Faculty of Nusa Cendana University, and Pandu Lestari Foundation), and Agricultural Department to identify, analysis, practise, and develop pratical technology in sustainable management of natural resources and environmental components.

Participatory model is conseptually much more better to be developed than just receiving technology package or intensive package to maintain food source during long period of dry season. This is mainly due to all the packages just overcome the problem in short periode of time and even create a dependence on certain intensive package. Moreover, the technology packages often do not in accordance with enviromental components where the technology should be practised easly by farmers. It is true that that packages offered with training to manage farming system, however, if some problems occur due to change of environemental compnents (physical, chemical, and biological characteristic of soil and pest and disease), the farmers did not able to overcome the problems. Meanwhile, Participatory model would practically take a longer time, however, the farmers, college students, and practitioners would some advantages such as

1) Community/ farmers may able to utilise wisely their natural resources and enviromental in supporting family food consumption, family income, and food security
2) Farmers and college students may able to identify weakness of natural resources and enviromental management, able to minimise those weakness by using practical technology, and even the weakness may able to develop become potential source
3) Lack of skill may able to improve during field learning process (envolving college students, lecturer, and field supervisor)
4) College student may able to interact directly to environment related condition (geo-physical, biology, socio-economic, socio-social, and public health) and community to aplicate their knowledge on field condition with complexity problems.

5) Colledge student may able to learn with community to develop potential and, minimise weakness of sources to be managed correctly based on sustainability and sustainable
6) The availability of food with various sources may able to support food security, familiy income, and family nutritions.

3.2 Problem Analysis

Table 2. result of identified problem analysed by fgd and ssi at terresterial, coastal, and mountainous enviroment at sub district of semau

Problem category	Identified problems		
	Vilage located at terresterial environment (Uitiuhana)	Vilage located at coastal environment (Akle)	Vilage located at mountainous environment (Uiboa)
Natural resources and physical enviroment	▪ Lack of land availability ▪ Burning ▪ Lack of water resource ▪ Ranch off	▪ Lack of land availability ▪ Burning ▪ Sedimentation ▪ Lack of water resource ▪ Coastal pollution tends to increase	▪ Erotion ▪ Burning ▪ Shifting cultivation ▪ Ranch off ▪ Lack of water resource ▪ Lack of land availability
Natural resources and biological enviroment	▪ Crop pest and desiase invasion ▪ Weed invasion ▪ Decreasing in biological diversity	▪ Decreasing in species and amount of fish catches ▪ Destructionof habitat for shelter ▪ Crop pest and disease invasion ▪ Weed invasion	▪ Decreasing of indigenous tree speciesl ▪ Increasing pest and disease invasiont ▪ Weed invasion
Socio economic aspect	▪ Increasing of population ▪ Lack of land productivity ▪ Improperly farming system model ▪ High dependence to local trader ▪ Increasing pest and disease invasiont	▪ Limitation of fishing eqipment ▪ Traditional technolofy of fish catching ▪ Low price ▪ Expensive supporting infrastrukture ▪ Increasing pest and disease invasiont	▪ Low of land productivity ▪ Improperly farming system model ▪ Increasing pest and disease invasiont ▪ Cattle destruction
Socio- culture aspect	▪ Low of land management ▪ Habit of burning ▪ Uncontrolled Slash and burn system ▪ Limitation of productive land	▪ Conflick of catching area ▪ Traditional technolofy of fish catching ▪ Lack of equipment and infrastrukture ▪ Low of land management	▪ Low of land management ▪ Burning system ▪ Uncontroled slash and burning system ▪ Limitation of productive land and livestock
Health and education aspect	▪ Lack of medical personnel ▪ Lack of clean water ▪ Lack of educational personnel ▪ Lack of environmental sanitation	▪ Lack of medical personnel ▪ Increasing of coastal pollution ▪ Lack of environmental sanitation ▪ Lack of clean water ▪ Lack of educational personnel	▪ Lack of medical personnel ▪ Lack of clean water ▪ Lack of educational personnel ▪ Lack of environmental sanitation ▪ Away lies the health service
Industry, service, and infrastructure	▪ Lack of skill ▪ Lack of community envolment ▪ Lack of telecomunications facilities ▪ Non-functioning cooperative ▪ Lack of local coordination ▪ Lack of clean water network	▪ Lack of skill ▪ Lack of community envolment ▪ Lack of telecomunications facilities ▪ Non-functioning cooperative ▪ Lack of local coordination ▪ Lack of clean water network ▪ Nopost harvest of fish industry ▪ Less management of means nautical	▪ Lack of skill ▪ Uneven electrical services ▪ Lack of community envolment ▪ Lack of telecomunications facilities ▪ Non-functioning cooperative ▪ Lack of local coordination ▪ Lack of clean water network

Result of identified problems which was analysed by FGD and SSI processes, were showed at Table 2 By using matric interaction technique among identified problems (Table 2), some main problems should be addressed to minimise destruction of natural resources and environmental componets that affect directly to soil and crop productivity, food consumption, food security, family income, family nutrients, and sustainability of enviroment. They were ***habit of burning, land degradation, shifting cultivation, ranch off, and improperly farming system***. Another problems that should be considered were ***lack of water resource, weed invasion, lack of biological diversity, lack of land productivity, and lack of productive land***

3.3 Potential Analysis

Potential analysis had been designed similar to problem analysis and it was combined by FGD and SSI to identify potential basic and potential derivates. Result of the studi was showed at Table 3

Table 3. result of identified potential analysed by fgd and ssi at terresterial, coastal, and mountainous enviroment at sub district of semau

Potential category	Identified potential		
	Vilage located at terresterial environment (Uitiuhana)	Vilage located at coastal environment (Akle)	Vilage located at mountainous environment (Uiboa)
Natural resources and physical enviroment	▪ Availability of agricultural land	▪ Availability of agricultural land	▪ Availability of agricultural land
Natural resources and biological enviroment i	▪ Ecotourism development ▪ Biological development	▪ Overflow source of fish ▪ Ecotourism development ▪ Less exploitation	▪ Ecotourism development
Socio economic aspect	▪ High level of productive age ▪ Availability of educational facilities	▪ Independence to food crops and estate crops product ▪ Availability of educational facilitie	▪ High level of productive age ▪ Availability of educational facilities ▪ Availability of land to develop farm
Socio- culture aspect	▪ Culture tourism ▪ Balancing of helong and Rote etnics	▪ Various etnics ▪ Culture tourism	▪ Various etnics ▪ Culture tourism
Health and education aspect	▪ Availability of health and educational services	▪ Availability of health and educational services	▪ Availability of health and educational services
Industry, service, and infrastructure	▪ Availability of employment ▪ Supporting from agricultural sector	▪ Road infrastructure ▪ Availability of employmen	▪ Availability of employmen ▪ Road infrastructure ▪ Supporting from agricultural sector

Result of interacted analysis showed that there were several priority potential source to be developed to support sustainability of agricultural production (plant, livestock, and fishery).

They were ***availability of agricultural land, unspoiled availability of coastal area, ecotourism development, and overflow source of fish***. All these potential source should be manage properly based on sustainability and sustainable point of view.

3.4 Interest Group Analysis

Interest Group Analysis was designed to identify which group of community was the most concerned with identified problems and potential to be managed in sustainable manner to ensure food security, family income, and family nutritions. Intererest Group was identified by analysis of situation run on FGD and SSI method. Result of the study was shown in detail at Table 4

Table 4. problems, need, and potential strategic groups in managing natural resources and environment at sub district of semau, district of kupang

Strategic Group	Problems	Needs	Potential
Small farmerl	• Matery of limited resources • Lack of skill • Low capital	• Empowerment • Venture capital assistance • Price stabilitya	• High amount • Political power • High participation level
Women and youth village	• Less educational accesstability • Less voting rights • Less empowerment	• Skill training • Recognition of rights • Opprtunity to take part	• High amount • Family labor
Professionals rural	• Less of amount • Limited support • Size wide services and hard	• Facility support • Oprational facility suport	• Special skill • Opportunity to influent • Got out access
Local middlemen	• Low capital • Less infrastructure	• Business smoothness • Empowerment	• Helping farmer need • Agricultural products market
Small businessman	• Low capitasl • Lack of skill • Lack of management capabilities • Limited market accessibility • Lack of competitiiveness	• Venture capital assitance • Promotion and marketing assistance	• Relatively high amounts • Create jobs • Contribution to local revenue
Local goverment	• Budget constraints • Waiting for instructions • Lack of skill	• Budget support • Cooperation with employers • Community support	• Authority planning and implementatio of development • Goverment administration

3.5 Referral program in natural resources and environmental management at semau island to support food security, family income, and family nutritions

Based on the results of problems and potential analysis natural resources and environmental components at Sub District of Semau, District of Kupang, some strategical programs are offered to manage sustainably natural resources and environment in supporting food security, family income and family nutrition, those are :

Enviromental Conservation : (1) development of indigenous trees which functioned as windbreak and firebreak, (2) improvement of biological diversity by developing tuber crops plant, jatropha, mutipurpose trees which are able to conserv soil and water, (3), development of integrated village forest, (4) development of integrated agricultural model, (5) development of land use model refered to soil and water conservation management, and (6) development of integrated agro and ecotourism model.

Sustainable Agriculture : (1) development of agroforestry system, (2) development of competitive commodities drought resistant such as various tuber crops species, (3) development of plant tuber crops as source of family nutritions, (4) evelopment of local cultivation model mainly concerned with land cultivation, pest and disese control, post harvest and storage, (5) development of drought resistent fodder, able to maintain soil moisture, and source of organic matter (*chromolaena odorata, mimosa sp*), (6) Development of integrated fishery model, (7) utilization of animal waste as an source of energy, and (8) development of indigenous plant as source of liquid organic fertilizer and water holding capacity

Human Resource Development : (1) development of central learning integrated management on food crops, horticultur, estate, and livestock, (2) development of central learning processing of alternative agricultural products particularly close relate to various tuber crops spcies, (3) utilizing the college students in the task of community Service Program, and (4) Excavation socio-cultural conditions.

It can be concluded that Participatory model in the term of field school and competitive research is promising model to be developed to improve sustainable natural resources and enviromental componets resulted in sustainability of soil and crops productivity, food security, family income, and family nutritions; Participatory model should be practised gradually and sustainably envolved directly all interest group (farmers, women, businessman, profesionals, practisioners, lectures, student, offical government, NGO); Food productivity, food security, family income, and family nutrition could be improve positively if farmers and other community being able to recognize, maintain, manage their natural resources and enviromental components correctly and wisely. It colud be done by learning together with nature inthe form of an integrated field school.

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THE VARIABILITY OF DUKU ACCESSIONS BASED ON THE CHARACTERS OF MORPHOLOGY, PHYSIOLOGY AND ANATOMY IN MUSI RAWAS REGENCY.

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ABSTRACT

The research was aimed to examine morphological, physiological and anatomical characters of duku accessions from three locations in Musi Rawas Regency. The experiment was carried out from February to April 2016 in Muara Lakitan District (Semanggus Lama Village) and in Muara Kelingi District (Bingin Jungut and Mambang Village), while plant physiology and anatomy analysis was carried out in the Laboratory of Plant Physiology, Department of Agronomy, Faculty of Agriculture, Sriwijaya University. The methodology used was direct survey and observation through purposive sampling with total 300 leaf samples. Results showed that variance was found in quantitative characters, while qualitative characters showed relatively similar results. Similarity analysis (dendogram) of 15 duku accessions in Musi Rawas Regency showed 67% of similarity level and a close genetic relationship.

Keywords: similarity level, duku, genetic relationship

INTRODUCTION

Duku (*Lansium domesticum* Corr.) is a perennial fruit tree originated from Indonesia. Duku plant in Indonesia has been widely distributed with production center areas located in Sumatra (South Sumatra, North Sumatra, West Sumatra and Jambi), Java (Central Java and Jakarta) and Kalimantan (West Kalimantan) (Direktorat Jenderal Bina Produksi Hortikultura, 2001). South Sumatra Province is one of the regions producing highly potential duku cultivar: Rasuan and Palembang cultivar which have distinctive taste and high economic value. Duku plantation area in South Sumatra is approximately 6,430.16 hectares with an average productivity of 5.7 tons / ha / year (Department of Agriculture and Horticulture of South Sumatra, 2002).

Based on the result findings from Lestari (2010), Musi Rawas is one of the centers of fruit production grown along Musi river basin where the fruit plantation are dominated by

annual crops such as durian, duku, and mango. Duku plant in Musi Rawas is the second famous commodity after durian, having a production value of 12 billion in 2008. Duku plant ecology is affected by river basin, shade and humidity, and grows well in double cropping plantation with durian. The largest duku plantation in Musi Rawas is located in Muara Kelingi and Kelingi Lakitan having 1,915 ha of planting area. Duku harvested area was 221 ha with 0.348 tons / ha productivity and 77 tons of production in the last 10 years (The Central Bureau of Statistics Musi Rawas, 2010).

Kartika et al. (2012) stated that the variability of plant morphological characters is necessary for tracing down the relations between ecology and vegetation analysis related to geography. Moreover, it also could be used the basis to determine cultivar categorization. Duku plants located in a same area are likely to have differences with each other mainly on the morphological appearance. Therefore, more studies related to the variability of potentially developed duku accession in Musi Rawas are needed to be heightened. The research was aimed to examine morphological, physiological, and anatomical characters of duku accessions in several locations of Musi Rawas Regency.

MATERIAL AND METHODS

The research was conducted from February to April 2016 in Musi Rawas Regency located in Semanggus Lama Village in Muara Lakitan District and in Bingin Jungut and Mambang Village in Muara Kelingi District. The analysis of physiology and anatomy was carried out in The Laboratory of Plant Physiology, Department of Agronomy, Faculty of Agriculture, University of Sriwijaya. The experimental tools used were Munsell Color Chart of Plant Tissue, cool box, scissors, higrometer, camera, compass, leaf area meter, microscope, measuring ruler, gauge, manual of Leaf Architecture, analytical balance, electrical oven, spectrophotometer, ladder, and plastic rope. While the materials included Aceton 80%, adhesive tape, filter paper, plastic bag, labelling paper, transparent nail polish, and duku plant samples.

Direct survey and observation through purposive samplings were carried out in three locations in Musi Rawas Regency in which there were five plant samples for each location. Data collection was performed following the direction of the wind and taken evenly for each sample. The total for leaf samples from each plant were 20 leaves. All data taken were presented in form of tables and figures.

Phenotypic analysis of variance was performed to examine the variability range of the observed characters by comparing the phenotypic characters and deviation standard. Genetic relationship was determined by using cluster method analysis UPGMA with NTSYS-pc 2.02 program (Rohlf, 1998). The value of phenotypic variance was calculated by the following formula : $\sigma_f^2 = \frac{\sum x_i^2 - (\sum x_{i\,\bar{}})^2 \quad /n}{(n-1)}$

The variability of observed characters was determined based on the criteria from Daradjat (1987) as below.

σ_f^2> 2.Sd : large phenotypic variability

σ_f^2< 2.Sd : narrow phenotypic variability

The working steps for the research included surveys and determining the locations, determining the plant samples, taking leaf samples, data collecting for morphological characters in the field, and anatomical and physiological analysis in the laboratory. The parameters were leaf and stem morphological characters, and leaf anatomical and physiological characters. The observed leaf morphological characters were leaf length, leaf width, leaf venation, shape of leaf surface / foliage type, shape of leaf edge, leaf color, leaf base, leaf tip and leaf surface area. While for the stem characters were plant height, stem girth, and branching shape. Leaf anatomical characters consisted of the numbers of adaxial and abaxial stomata, and stomata shape. Leaf physiological characters consisted of leaf chlorophyll, leaf sucrose, and total leaf nitrogen.

RESULTS AND DISCUSSION

Morphological Characteristics of Duku Plant

The quantitative morphological characters of duku plants showed similarity for all observed characters, such as leaf length and leaf width. The variance was found in plant height, leaf area and stem girth as given in Table 1. From the table, it was found that the accession of SL 3 had the longest leaf with 19.76 cm and the shortest was BJ 1 with 15.5 cm. The average of leaf length was 17.18 cm. For leaf width, the widest leaf was also SL 3 with 10.08 cm and the narrowest was BJ 4 with 7.3 cm, while the average was 8.18 cm. Leaf area of BJ 1 was 185.188 cm^2 which was the highest among others and the smallest was DM 4 with 139.704 cm^2 and the average was 160.185 cm^2. BJ 1 also had the tallest plant height with 20 m and the shortest was DM 5 with 8 m and the average of plant height was 13.5 m.

The largest stem girth was in SL 4 with 167 cm and the smallest was DM 5 with 45 cm. The average of stem girth was 116.6 cm.

Table 1. The quantitative morphological characteristics of duku plant.

Accession	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Plant height (m)	Stem girth (cm)
SL1	17.09	8.96	148.014	20	156
SL2	16.20	7.83	148.959	18	164
SL3	19.76	10.08	175.395	11	142
SL4	16.74	8.24	158.178	16	167
SL5	15.83	7.62	159.886	10	108
SL average	17.12	8.54	158.086	15	147.4
BJ1	17.85	8.55	185.188	10	90
BJ2	15.50	7.46	155.370	20	154
BJ3	17.80	8.76	168.794	13	93
BJ4	17.26	7.30	141.657	17.5	147
BJ5	18.56	8.47	164.290	15	128
BJ average	17.39	8.10	163.059	15.1	122.4
DM1	16.44	7.48	169.236	14	102
DM2	17.38	7.75	149.376	8	73
DM3	17.78	8.13	176.630	10	86
DM4	16.74	7.99	139.704	12	94
DM5	16.95	8.27	162.112	8	45
DM average	17.05	7.92	159.411	10.4	80
Total average	17.18	8.18	160.185	13.5	116.6

Note : SL : Semanggus Lama, BJ : Bingin Jungut, DM : Desa Mambang

The qualitative morphological character of duku plants showed similarities in all observed characters, such as leaf venation, leaf shape, leaf edge, leaf base, leaf tip and branching pattern. While the variance only occurred on leaf color as presented in Table 2.

Table 2. Qualitative morphological characters of duku plant

	Leaf morphology						Stem morphology
Accession	Leaf venation	Leaf shape	Leaf edge	Leaf color	Leaf base	Leaf tip	Branching type
SL1	Pinnate	Eliptic	Entire	7,5 GY (4/6)	Complex	Accuminate	Monopodial
SL2	Pinnate	Eliptic	Entire	7,5 GY (3/4)	Complex	Accuminate	Monopodial
SL3	Pinnate	Eliptic	Entire	7,5 GY (3/4)	Complex	Accuminate	Monopodial
SL4	Pinnate	Eliptic	Entire	5 GY (4/8)	Complex	Accuminate	Monopodial
SL5	Pinnate	Eliptic	Entire	7,5 GY (4/8)	Complex	Accuminate	Monopodial
BJ1	Pinnate	Eliptic	Entire	7,5 GY (4/4)	Complex	Accuminate	Monopodial
BJ2	Pinnate	Eliptic	Entire	7,5 GY (4/4)	Complex	Accuminate	Monopodial
BJ3	Pinnate	Eliptic	Entire	7,5 GY (4/6)	Complex	Accuminate	Monopodial
BJ4	Pinnate	Eliptic	Entire	7,5 GY (4/4)	Complex	Accuminate	Monopodial
BJ5	Pinnate	Eliptic	Entire	7,5 GY (3/4)	Complex	Accuminate	Monopodial
DM1	Pinnate	Eliptic	Entire	7,5 GY (4/6)	Complex	Accuminate	Monopodial
DM2	Pinnate	Eliptic	Entire	7,5 GY (3/4)	Complex	Accuminate	Monopodial
DM3	Pinnate	Eliptic	Entire	7,5 GY (4/6)	Complex	Accuminate	Monopodial
DM4	Pinnate	Eliptic	Entire	7,5 GY (4/4)	Complex	Accuminate	Monopodial
DM5	Pinnate	Eliptic	Entire	7,5 GY (4/4)	Complex	Accuminate	Monopodial

Note : - GY/Green Yellowish (Munsell book scale)
- Characteristics were determined based on Manual of leaf architecture (Ash et al., 1999).

All duku accession taken as samples from three sites in Musi Rawas Regency had relatively similar elliptical leaf shape. This elliptical leaf shape had the ratio of length and width as 1.5-2 : 1 and the petiole was in the base of lamina. The pinnate leaf had a single venation. While the entire leaf had an edging shape in form of non-fibreless thin segment. Leaf color differed among locations in which SL was 7,5 GY (3/4), BJ 7,5 GY (4/4), and DM 7,5 GY (4/4) and 7,5 GY (4/6). Leaf base was complex and leaf tip was acuminate. Monopodial branching shape was branching type in which the main stem was more visible, bigger and longer than its branches, and branching direction tend to go upward.

Leaf Physiological Characteristics

Leaf physiological characteristics could be evaluated from leaf chlorophyll content, leaf sucrose and leaf total N. The highest leaf chlorophyll was found in DM 5 with 27.665 mg/l and the lowest was 13.067 mg/l in BJ, while the average was 19.91 mg/l. The highest leaf sucrose was 3.531% in BJ 2 and the lowest was 1.671% in DM 4. Leaf sucrose average was 2.78%. The highest leaf total N was found in DM 3 with 3.08% and the lowest was in SL 1 with 0.98%. The average of leaf total N was 1.98%. All data of leaf physiological characters were given in Table 3.

Table 3. Leaf physiological characters

Accession	Leaf chlorophyll (mg/l)	Leaf sucrose (%)	Leaf total N (%)
SL1	20.69	2.65	0.98
SL2	16.95	2.95	1.82
SL3	16.26	2.84	2.10
SL4	14.67	2.74	1.96
SL5	20.05	2.47	2.24
SL average	17.72	2.73	1.82
BJ1	17.04	2.91	2.52
BJ2	26.27	3.53	1.96
BJ3	21.00	3.37	1.96
BJ4	20.11	3.33	1.26
BJ5	13.06	3.52	2.1
BJ average	19.49	3.33	1.96
DM1	18.65	1.92	1.12
DM2	23.86	2.69	2.24
DM3	19.17	2.31	3.08
DM4	23.26	1.67	2.38
DM5	27.66	2.85	2.10
DM average	22.52	2.28	2.18
Total average	19.91	2.78	1.98

Anatomical Characteristics

Leaf anatomical characteristics were obtained from the number of adaxial and abaxial stomata, and stomata shape as given in Table 4. The highest number of adaxial stomata was found in BJ 1 with 10.7 and the lowest was 6.2 in SL 2, while the average was 9.3. BJ 3 was the accession with the lowest number of abaxial stomata which was 26.7 and the lowest was in DM 5 with 15.2. The average of abaxial stomata was 20.1. Stomata shape for all accession was Anomocytic.

Phenotipic Analysis of Variance

By comparing phenotipic variability to standard deviation of nine parameters to evaluate the variability range, it was found that there were five narrow (similar) quantitative parameters of duku accessions which were leaf length, leaf width, number of adaxial stomata, leaf sucrose, and leaf total N.

Table 4. Leaf Anatomical Characteristics

Accession	Number of adaxial stomata	Number of abaxial stomata	Stomata shape
SL1	11.5	25.7	Anomocytic
SL2	6.2	17	Anomocytic
SL3	12	21.7	Anomocytic
SL4	9	21.7	Anomocytic
SL5	9.2	18.5	Anomocytic
SL average	9.5	20.9	Anomocytic
BJ1	10.7	20	Anomocytic
BJ2	8.7	21	Anomocytic
BJ3	9.7	26.7	Anomocytic
BJ4	9.7	20	Anomocytic
BJ5	9	19.5	Anomocytic
BJ average	9.5	21.8	Anomocytic
DM1	8.7	17.7	Anomocytic
DM2	8.5	19.5	Anomocytic
DM3	9.7	20.7	Anomocytic
DM4	10.5	16.2	Anomocytic
DM5	7.7	15.2	Anomocytic
DM average	9.02	17.8	Anomocytic
Total average	9.34	20.1	Anomocytic

Note : Stomata shape was determined based on the Manual of leaf architecture (Ash *et al*. 1999).

While large (varied) characters were represented by leaf area, plant length, stem girth, number of abaxial stomata, and leaf chlorophyll as shown in Table 5. There were no variance found for qualitative morphological and anatomical characters of duku accessions.

Table 5. Phenotipic Variability of Duku Accession in Musi Rawas Regency

Characters	Average	Phenotipic variability ($\sigma^2 f$)	Standard Deviation (2sd)	Criteria
Leaf length (cm)	17.19	1.71	2.16	Narrow
Leaf widht (cm)	8.19	0.51	1.43	Narrow
Leaf area (cm ^{2})	160.18	177.47	26.64	Large
Plant height (m)	13.50	277.98	105.33	Large
Stem girth	116.60	1390.25	74.57	Large
Number of adaxial stomata	9.41	2.08	2.88	Narrow
Number of abaxial stomata	20.23	10.21	6.39	Large
Leaf chlorophyll (mg/l)	19.91	16.86	8.21	Large
Leaf sucrose (%)	2.78	0.29	1.08	Narrow
Leaf total N (%)	1.98	0.29	1.08	Narrow

Note : Variability value used the method of Daradjat (1987)

Genetic Relationship among Accessions

The analysis of genetic relationship among duku accessions was carried out based on 17 characters of the morholophy, physiology and anatomy of duku plants.

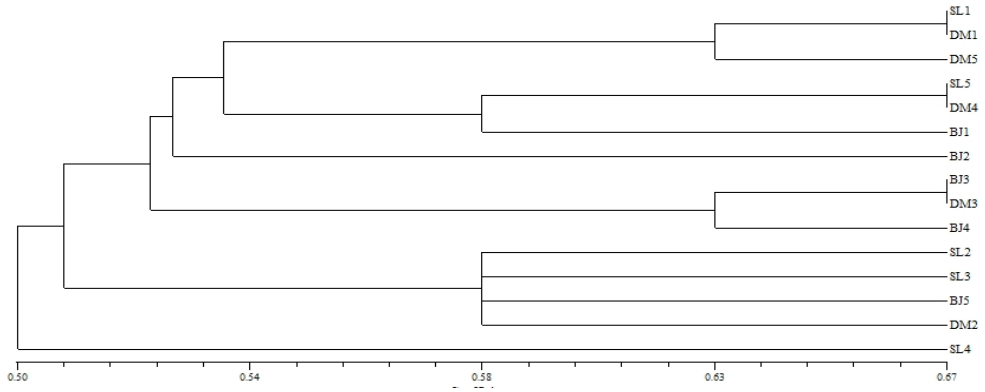

Fig. 1. The dendogram of genetic relationship among duku accessions in Musi Rawas Regency

The relation was presented by using the dendogram (Fig. 1) and showed the minimum 50 % of similarity index. There were two groups of duku accessions. First was the accession originated from SL, BJ and DM. The second group was only the accession from SL. The nearest genetic relation was found in 67% of similarity index from SL1 – DM 1, SL 5 – DM 4 and BJ 3 – DM 3. The higher similarity index, the closer genetic relationship among the accessions.

The research of duku accessions variability based on morphological characters were categorized into two: quantitative and qualitative for both leaf and stem. In general, the

quantitative characters of both leaf and stem morphology showed more variations compared to qualitative characters. Ferita *et al*. (2015) stated that quantitative character was the character affected by several genotypic factors (internal factors), however, the phenotypic factors(external factor) still had more significant impact. Leaf quantitative characters included leaf length, leaf width and leaf area. While for the stem, they were plant height and stem girth. According to Satria *et al*. (2008), the variation of leaf measurements (length, width and area) was due to the variations in environment, such as light, water availability, and soil fertility.

The qualitative characters of duku leaf morphology in Musi Rawas Regency were indicated by having elliptical leaf shape, pinnate leaf venation, entire leaf edge, complex leaf base and accuminate leaf tip. While the qualitative characters of stem morphology were determined by the monopodial branching type. The variations on qualitative characters of plant morphology were mostly influenced by genetic factor causing the similarity among the accessions (Suryani and Nurmansyah, 2009) since the genes had the ability to surpress the environmental factor.

Leaf physiological characters of duku plants in Musi Rawas Regency were characterized by the leaf chlorophyll. The highest leaf chlorophyll was found in Mambang Village with 27.66 mg/l and the lowest was 13.06 mg/l in Bingin Jungut Village, and the total average was 19.91 mg/l. This research resulted different leaf chlorophyll for each duku accession causing the difference in photosynthetic activity and the yield (Ristiawan, 2011). Leaf chlorophyll could also be detected from the leaf color in which the greener leaf color, the higher leaf chlorophyll.

The highest leaf sucrose content was obtained from duku accession in Bingin Jungut Village with 3.53%, while the lowest was 1.67% obtained from Mambang Village and the total average was 2.78%. Both Bingin Jungut and Mambang Village were in the same district, Muara Lakitan District. Leaf sucrose affected the forming of carbohydrate in plants (Sholikhah *et al*., 2015). Sucrose was the main product resulted from photosynthesis and could be translocated to all plant's organs through phloem to support plant growth and development.

Duku accession in Mambang Village had the highest leaf total N which was 3.08%, while the lowest was in Semanggus Lama Village with 0.98%. Total average of leaf N was 1.98%. High leaf total N in Mambang Village was due to high light intensity. Nitrogen was

the main element composing chlorophyll which functioned as light interceptor for the photosynthesis (Ristiawan, 2011). So that the increase in leaf chlorophyll would affect leaf total N since N was the main element in chlorophyll (Hernita *et al*., 2012). Physiological characters of duku plants were determined by light intensity, temperature, humidity and soil fertility.

Leaf anatomical characters were identified by the number of both adaxial and abaxial stomata, and stomata shape. The average of adaxial stomata was 9.34 and abaxial stomata was 20.16. Smaller number of adaxial stomata was plant adaptation method to minimize water loss, wind and sunlight heat. All accessions had the same Anomocytic stomata shape, where the guard cells were surrounded by several certain cells with similar shape and size (Damayanti, 2007). While Polycytic type was defined as stomata with the guard cells surrounded by 5 cells with indifferent shape with other epidermis cells. The shape of stomata was related to the transpiration rate and also to sunlight intensity.

The results of phenotypic variability analysis showed two type of variabilities: narrow and large. Narrow variability (similar) was obtained from leaf length, leaf width, number of adaxial stomata, leaf sucrose, leaf total N, leaf shape, leaf venation, leaf edge, leaf base, leaf tip, branching type, and stomata shape. Narrow variability showed the impact of environmental factor to plant performance (Mangoendidjojo, 2003; Ferita *et al*., 2015). Large variability was identified from leaf color, leaf area, plant height, stem girth, number of abaxial stomata, and leaf chlorophyll. Large variability showed the role of genetic factor in the variability of plant characters (Satria *et al*., 2008). The phenotypic variability occurred due to the interaction between the genetic and environmental factor.

Similarity analysis (dendogram) of 15 duku accessions in Musi Rawas Regency had the highest genetic relation index of 67% and 50% of the variability level. Similarity analysis was used to determine the range of similarity relationship among plants by using the morphological characters (Ferita *et al*., 2015). Duku accessions in this research were originated from three different locations: Semanggus Lama, SL; Bingin Jungut, BJ; and Mambang, DM. Aryanti (2015) stated that plants cultivated in the same location would have a differentiation in the growth and development due to environmental factor expressed by their phenotypic appearance.

The conclusions for the research were as the following.

1. The highest similarity level of duku accessions in Musi Rawas Regency reached 67% with 50% of variability.
2. The similarity of morphological characters was due to plant genetic factor, while the variability was caused by environmental factor.
3. Leaf physiological characters of duku accessions had high leaf chlorophyll, leaf sucrose, and leaf total N which were affected by sunlight intensity, temperature, humidity, and soil fertility.
4. The characters of duku anatomy were identified by having many stomata in anomocytic shape.

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SUITABILITY OF LAND AREA FUNCTION TO THE EXISTING LAND USE OF BLONGKENG SUB WATERSHED, JAVA, INDONESIA

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ABSTRACT

The Government of Indonesia has the rule to state the land area function, however the existing land use could be improper to its function. The objective of this research is to find out the variation of the existing land use of the Blongkeng sub watershed, to study the land area function, and finally to evaluate the suitability of the existing land use with the land area function. This research was carried out in Blongkeng sub watershed. Observatory research method was applied and the parameters collected include slopes, soil type, and rainfall using maps. GIS was applied and data were analyzed using quantitative descriptive. Blongkeng sub watershed has the area of 7,498.64 ha and consists of 92 land units and 9 land use types includes: mixed cropping, National Park, dry field, water, shrubs, settlement, grass, rice field, and dry rice field. The land area function is composed: 397 ha of protected (5.29%); 1,292.56 ha of buffer (17.24%); 1,265.51 ha of annual plant silviculture (16.88%), and 4,543.58 ha of perennial crop and settlement (60.59%). The existing land use which is improper with the land area function was found in all land area function. Most of the existing land use of Blongkeng sub watershed area is suitable to the function of the land (83.65%) and the rest of the area (16.35%) is improper.

Keywords: land area function, suitability, Blongkeng

INTRODUCTION

The high human dependency on land result in the more land is required to fulfill human's need, the more intensive and the more improper land use. The increase number of population will impact on the increase food, clothes, and places for human living. Watershed is the area which has the natural boarder. Naturally, the watershed quality is influenced by the biophysical characteristics such as soil, relief, topography, climate, water, and vegetation (Tan, 1991). However human's activities in using the land cause the disturbance of watershed

equilibrium. Land exploitation creates problems such as: floods in the rainy season and drought in the dry season; the decrease of discharge; erosion and sedimentation; and landslides. As a consequence, these hazards create the reducing of land productivity and the lack of water along the year (Komaruddin, 2008).

Generally, the usage of the land will result in the land use change which usually does not followed by the techniques to combat land degradation. Even Butchart et al. in Bailey et al. (2016) mentioned that globally, there has been a decline in biodiversity over the last four decades, attributed in large part to habitat fragmentation and land conversion. Based on the above explanation, it is very important to study about how the existing land use is improper to its function. The Government of Indonesia has the rule to state the area of the watershed, composed 4 types, these are: protected zone, buffer zone, annual plant silviculture, perennial crops and settlement. Although there is a strict rule stated, but not all of the existing land use fits or suitable to the function of the land (zone).

The goals of this research was to quantify the existing land use of Blongkeng sub watershed, to study the distribution of the function of the land (zone) and to evaluate the land use which is improper to the function of the land (zone).

MATERIALS AND METHODS

Land suitability was discussed in a significant amount of literature. One approach to establishing appropriate land use criteria is the construction of suitability analysis. Suitability techniques are essential for informed decision making (Steiner et al, 2000). According to Rawat et al (2010), the synthesis of thematic layers results different terrain units of land suitability class which is important for planning, land use, and land management. Moreover, Vink (in Steiner, 2012) defines soil suitability as physical suitability of soil and climate, or production of a crop or group or sequence of crop, or for other defined uses or benefits , within a specified socio economic context but not considering economic factors specified.

This research was carried out in Blongkeng sub watershed. Data collected includes primary and secondary data. Observatory research method was applied and the parameters collected include slopes, soil type, and rainfall using maps. GIS was applied and data were analyzed using quantitative descriptive.

The site assessment was started by making the land unit, 3 maps (soil type, slopes, and land use maps) were overlapped. At every land units then was evaluated by using the

Government of Indonesia's rule: Ministry of Agricultural Decree number 837/Kpts/Unit/11/1980 and 683/Kpts/Um/8/1981. There are 3 parameters considered in this method includes: slopes, soil type, and rainfall. These 3 parameters were classified and scored as seen in Table 1.

Table 1. Parameters and scores of the function of the land

1	Slope	Slope of (%)	Class	Score
		0-8	Flat	20
		8-15	Undulating	40
		15-25	Moderate steep	60
		25-45	Steep	80
		>45	Very steep	100
2	Soil	Soil type	Class	Score
		Alluvial, Gley, Planosol, Grey Hydromorph, Lateritic, Ground water	Not sensitive	15
		Latosol	Less sensitive	30
		Brown Forest Soil, Non Calcic	Moderate Sensitive	45
		Andosol. Lateritic	Sensitive	60
		Grumosol, Podsolic Regosol, Lithosol, Organosol, Rendzina	Very sensitive	75
3	Rainfall	Rainfall (mm/yr)	Class	Score
		1,500-2,000	Very low	10
		2,000-2,500	Low	20
		2,500-3,000	Moderate	30
		3,000-3,500	High	40
		3,500-4,000	Very high	50

After scoring of the 3 parameters, then the sum of those was calculated and indicated the zone type by using Table 2.

Table 2. Criteria of the function of the land.

Number	Function of the land (zone)	Total score
1	Protected	>175
2	Buffer	125-175
3	Annual plant Silviculture	<125
4	Perennial Crop and settlement	<125, slopes: ≤ 8%

To analyze the land use which is improper to the function of the land, the map of the zone was overlapped with the existing land use. Then, to evaluate the resulted map, matching analysis was applied.

RESULTS AND DISCUSSION

Blongkeng sub watershed has the area of 7,498.64 ha and consists of 92 land units and 9 land use types includes: mixed cropping, National Park, dry field, water, shrubs, settlement, grass, rice field, and dry rice field.

The synthesis of the 3 parameters that are slope, soil type and rainfall in Blongkeng sub watershed results into 4 land area functions: 397 ha of protected zone (5.29%); 1,292.56 ha of buffer zone (17.24%); 1,265.51 ha of annual plant silviculture zone (16.88%), and 4,543.58 ha of perennial crop and settlement zone (60.59%). The protected zone is the smallest, by contrary, this area has the most important function for controlling the other zone. The buffer zone and the annual plant silviculture zone each has an area of around 17% of the watershed area, although both the buffer zone and annual plant zone also have a significant role in protecting the land from erosion, sedimentation and water conservation. The largest area is perennial crops and settlement zone, it means that the human dependency on the land is very high, where they use most of the land for fulfilling their food and settlement. To evaluate the existing land use, this map was overlapped with the function of the land and the result is below (Table 3)

Table 3. The evaluation of the function of the land and land use

No.	Function of the land (Zone)	Land use	Area (Ha)	Suitable (S)	Not Suitable (TS)
1	Perennial crop and settlement	Dry rice field	594.48	S	
2	Perennial crop and settlement	Water	103.13	S	
3	Perennial crop and settlement	Mixed cropping	228.79		TS
4	Perennial crop and settlement	Settlement	1141.07	S	
5	Perennial crop and settlement	Dry field	25.07	S	
6	Perennial crop and settlement	Building	0.49	S	
7	Perennial crop and settlement	Rice field	2450.56	S	
8	Annual plant silviculture	Water	14.61		TS
9	Annual plant silviculture	Shrubs	12.37		TS
10	Annual plant silviculture	Mixed cropping	217.41	S	
11	Annual plant silviculture	Settlement	85.65		TS
12	Annual plant silviculture	Grass	42.74		TS
13	Annual plant silviculture	Rice field	604.25		TS
14	Annual plant silviculture	National park	196.54	S	
15	Annual plant silviculture	Dry field	91.95	S	
16	Protected	Shrubs	0.04		TS
17	Protected	Grass	0.40		TS
18	Protected	National park	392.85	S	
19	Protected	Dry field	3.71		TS
20	Buffer	Dry rice field	4.34		TS
21	Buffer	Water	0.51	S	
22	Buffer	Shrubs	24.02		TS
23	Buffer	Mixed cropping	268.90	S	
24	Buffer	Settlement	24.23		TS
25	Buffer	Grass	15.20		TS
26	Buffer	Rice field	25.37		TS
27	Buffer	National Park	789.54	S	
28	Buffer	Dry field	140.45		TS
	Total (Ha)		**7,498.64**	**6,272.47**	**1,226.17**
	(%)		**100**	**83.65**	**16.35**

The above table shows that most of the existing land use of Blongkeng sub watershed area is suitable to the function of the land (83.65%) and the rest of the area (16.35%) is improper. The evaluation also shows that the improper land use happens at all of the function of the land (zone). Furthermore, it can be noticed that the people has converted the protected, buffer and annual plant silviculture zones to the land use that its yields potential for covering their foods and for their settlements. It can be seen that they use those three zones for rice field, settlements, and dry field. According to Nugraha (2009), the improper land use can

cause the the land degradation and environmental disturbance. So, although only around 17 % of the watershed area is classiified as improper land use, efforts in reducing the ecological hazard should be implemented in Blongkeng sub watershed.

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TOTAL PHENOLIC, ANTIOXIDANT ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES OF WAXY PIGMENTED AND NON-PIGMENTED RICE

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ABSTRACT

This study determined the physicochemical properties, total phenolic content, and antioxidant activity of waxy pigmented and non-pigmented rice varieties. The results show that, waxy pigmented rice has 2.1% for amylose, 8.31% for crude protein, 2.67 % crude fat, 0.77 % crude fiber, 0.59 % crude ash, 7 mm for length, 1.8 mm for width, 16.57 g weight/1000grain, 2047 ug g^{-1} for total phenolic content, 12.54 % for DPPH-2,2-diphenyl-1-picrylhydrazyl (DPPH) and 0.138 % Copper reduction antioxidant capacity (CUPRAC) compared to waxy non-pigmented rice but the 86.69 % of starch and 11.7 % of moisture content was lower compared to waxy non-pigmented rice. The result of total phenolic content and antioxidant activity is one way of utilizing the local drought resistant crops for nutritious and safe products in the promotion of food security in the country.

Keywords: Waxy pigmented and non-pigmented rice, proximate properties, total phenolic content, and antioxidant activity

INTRODUCTION

In the mid-1990s food security was recognized as a significant concern, spanning a spectrum from the individual to the global level. However, access now involved sufficient food, indicating continuing concern with protein-energy malnutrition. But the definition was broadened to incorporate food safety and also nutritional balance, reflecting concerns about food composition and minor nutrient requirements for an active and healthy life.

At present, **food security** does not only mean enough quantity of food to feed the population but exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy

life. *Domneub* is a sweet rice or glutinous rice variety in Cambodia which has two types; the white waxy non-pigmented rice (*Domneubsor* in Khmer) and black waxy pigmented rice (*Domneobkhmau* in Khmer). *Domneubsor* is used throughout the country for production of rice cake, dessert, *tape* (saccharified rice), among others, while the *Domneubkhmau* is used for traditional medicine processing by fermentation, fish fermentation, *tapekhmau* (black saccharified rice). In this study, *Domneubsor* and*Domneubkhmau*were utilized for rice wine fermentation. However, there is very limited information about the benefits and composition properties in both rice. Moreover, the data on the physicochemical properties of these rice varieties after processing were also limited.

Pigmented or colored rice is characterized by its grain with red brown or dark purple color covering layers. Pigments, which are located in the aleurone layer of rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids (Yawadio *et al.,* 2007). The phenolic compounds have been found as a major active component for antioxidation (Iqbal *et al.,* 2000; Zhang *et al.,* 2006; Yawadio *et al.,* 2007; Tabart *et al.,* 2009). Antioxidative activity of pigmented rice has been reported by Zhang *et al.* 2006; Nam *et al.* 2006 and Chung and Shin 2007). However there is no specific information on Total phenolic content and Antioxidant activity of *Domneubsor* and *Domneubkhmau* grown in Cambodia.

Thus, this study evaluated the physicochemical properties, total phenolic content and antioxidant activity of the waxy pigmented and non-pigmented Cambodian rice varieties.

MATERIALS AND METHODS

Experimental Materials

Waxy pigmented and non-pigmented rice varieties were purchased from Cambodia and transported to the Philippines. Waxy pigmented rice was obtained from Trapeang Ta Moung Village, Prey Sleok Commune, Treang District, Takeo Province known as *angkordomneubkhmao* or black sticky rice. The waxy non-pigmented rice was collected from Teamchas Village, Kompongsvay Commune and District, Kompong Thom Province, Cambodia locally known as *angkordomneubsor* or white sticky rice.

Amylose Content (AC)

The amylose content of the rice samples was determined by Iodine-Amylose colorimetric assays for apparent AC method following the procedure of Juliano *et al.* (2012). Waxy pigmented and non-pigmented milled rice flour (100mg) were wetted with 1.0mL of 95% ethanol and swirled carefully to disperse clumps. The ethanol-wetted flour was

dispersed in 1 N NaOH (9.0mL) in a 100mL volumetric flask and allowed to stand overnight. The mixture was made up to 100mL with distilled water, mixed and a 5mL aliquot (0.09 N NaOH) was placed in a 100mL volumetric flask with ≈ 50mL of distilled water. Then, 1.0mL of 0.9 N NH_4Cl was added, followed by 2mL of 0.15% iodine in 1.5% KI, and the solution was made up to volume with distilled water to obtain a stable deep-blue color with the least amount of interference from amylopectin (waxy starch produces a greenish tinge). The absorbance of the color was read at 620 nm within 20-60 minutes and its stability and pH were measured. AC was calculated from standard curves based on potato amylose V (Avebe) alone (0, 5, 10, 15, 25 and 35 mg/100mL) of 0.09 N NaOH.

Rice varieties were classified into five groups according to their amylose content: waxy (0.0-2%), Very low amylose (2.1–9.9%), low amylose (10.0–17.0%), intermediate amylose (17.1-21.9%) and high amylose (≥22.1%) (Rice Chemistry and Quality Laboratory, Philippine Rice Research Institute, University of the Philippines Los Banos 2014).

Physicochemical Analysis

The physicochemical properties of both the raw materials (waxy pigmented and non-pigmented rice) inrice wine resulting from traditional and multi-parallel fermentation using the developed starter culture were analyzed.For the raw materials, one hundred grains of each rice variety were taken randomly and the length and width were measured by using a micrometer. Representative samples of waxy pigmented and non-pigmented rice varieties (50g) were drawn randomly and thousand grain weights was recorded in grams/1000 kernel by counting grains and weighing on an electric balance described by AACC (2000). Protein content was determined using a Kjeldahl digestion system based on the Association of Official Analytical Chemists (AOAC) method (2000). Crude fat was determined by extraction with hexane for 6 h using a Soxhlet apparatus. Ash content was determined by burning in a muffle furnace at 550^{o}C for 3 h according to the AACC (2000). The moisture content in each sample was determined by drying 4g sample in an air forced draft oven maintained at a temperature of 105±5^{o}C according to the procedure described in AACC (2000) method No. 44-15A. For the determination of fiber content, the rice samples were digested with 1.25% H_2SO_4 followed by 1.25% NaOH solution and crude fiber content was determined according to AACC (2000) method No.32-10.

Determination of Total Phenolic Content

The total phenolic content was determined by Folin-Ciocalteu method according to the procedure of Teresa Escribano-Bailón *et al.*(2002). The sample was diluted with water and to 0.5 mL of the diluted samples, 0.5 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich) and 0.5% Na_2CO_3 were added. After standing for 5 minutes, 5 mL distilled water were added. The absorbance readings were measured at 720 nm (Shimadzu UV-1601 spectrophotometer) with water plus reagent as blank sample. Total phenolic content was computed in a standard curve with gallic acid as reference phenol. The results were expressed as gallic acid equivalents (GAE mL^{-1}).

Determination of Antioxidant Activity (DPPH Radical Scavenging Method)

The antioxidant activity of polyphenols is measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Ribeiro *et al.*(2008) with some modifications with synthetic antioxidants butylatedhydroxy-anisole (BHA) and butylatedhydroxy-toluene (BHT) as reference antioxidants.

Five mL (5 mL) of 0.1mM DPPH were pipetted in test tubes and added with 100 uL of the standards and sample. The mixture was vortexed and allowed to stand for 20 minutes. The control consisted of 100 uL methanol and the reagent served as control. The absorbance was read at 517 nm with water to zero the instrument. The percentage of inhibition was expressed using the following equation:

$$\%Inhibition = \left[\frac{A_{control} - A_{Sample}}{A_{Control}}\right] x\ 100$$

Copper reduction Antioxidant Capacity (CUPRAC) Test

Copper reduction antioxidant capacity (CUPRAC) test is a variant of FRAP test using Cu instead of Fe. The assay is based on the reduction of Cu^{+2} to Cu^{+1} by the combined action of all antioxidants (reducing agents) in a sample. The Cu^{+1} then forms a yellow colored complex with neocuproine (2,9-dimethyl-1,10-phenanthroline) which absorbs at 450 nm. Therefore, the higher the absorbance at 450 nm, the higher the reduction of copper by the antioxidants, the higher the antioxidant capacity, the better the health beneficial quality of foods. One mL (1 mL) of $CuCl_2$, 1.0mL of NH_4AC, 1.0 mL of neocuproine, 0.5 mL of antioxidant and 0.6 mL of water were pipetted in test tubes. The concentration of antioxidants was prepared using different concentrations of 5, 10, 15, 20 and 25 ug GAEml^{-1}. The mixture was vortexed and

allowed to stand for 30 minutes and then read at 450 against a blank sample consisted of water and the reagents (Alpinar *et al.,* 2009).

RESULTS AND DISCUSSION

Physicochemical Properties of the Waxy Pigmented and Non-pigmented Rice

Short-grained varieties of rice are considered best for *sake (Japanese wine)* manufacture and long-grained varieties are considered desirable. Types of rice-long grain, medium grain and short grain are based on the length: width ratio of kernels of rice grains that are unbroken (Matz, 1991). Table 1 shows the comparison of the physical and chemical properties between the rice varieties (*Domneupkmau and Domneupsor*) used in the study for production of rice wine. Both varieties fall under long-grained types. Their length ranges from 6 to 7 mm while the width is from 1.5 to 1.8 mm.

The amylose content of rice is one of the most important criteria of rice quality in terms of cooking and pasting properties (Adu-Kwarteng *et al.,*2003). The rice varieties tested were observed, of which non-pigmented rice can be classified as the variety with "very low amylose" content than waxy pigmented rice. According to the classification of Rice Chemistry and Quality Laboratory, Philippine Rice Research Institute, University of the Philippines Los Banos, waxy rice (0.02-2.0%) with very low amylose content (2.1-9.9%) are sticky after cooking. Thus, waxy pigmented and non-pigmented rice samples are classified as varieties with "very low amylose" content and "waxy". In addition, amylopectin predominates in waxy rice whereas amylose is much more predominant in non-waxy rice. Amylose is more soluble in water than amylopectin and their ratio influences cooked rice properties.

Waxy non-pigmented rice has high moisture content (12.64%) compared to waxy pigmented rice (11.7%). In general, except for starch and moisture content, all mean values were found higher in waxy pigmented than non-pigmented rice variety. Protein influences the nutritional quality of rice. In this study the protein content was appreciably high (6-8%) for both tested varieties. According to Nunokawa (1972), most rice varieties for *sake* making contain 7.0 to 9.0% crude protein.

Total Phenolic Content in Waxy Pigmented and Non-Pigmented Rice

The total phenolic content of the waxy pigmented and non-pigmented rice samples expressed in milligram of gallic acid equivalent (GAE 100^{-g}) dry sample is presented in Table

1. It can be seen that the total polyphenol content of the different types of waxy rice varies significantly from each other. Results revealed that the waxy pigmented rice exhibited a significantly higher total polyphenol content of 2074 ug GAE g^{-1} compared to that of the waxy non-pigmented rice of 134 ug GAE g^{-1} db. Waxy pigmented rice had higher total phenolic content than waxy non-pigmented rice. The waxy pigmented rice had higher phenolic content than the red and black rice varieties with mean phenolic contents of 470.1 and 1055.7 mg/100g, respectively, as reported by Goffman and Bergman (2004) and Shen*et al.* (2009). Polyphenols are the most effective antioxidative constituents in plant products consumed (Escribano-BailÓn *et al.,* 2004).

Antioxidant Activity in Waxy Pigmented and Non-Pigmented Rice

DPPH radical scavenging activity. DPPH is a free radical that accepts an electron or hydrogen radical to form a more stable compound. DPPH assay is based on the decrease in purple color of the DPPH solution when the nitrogen atom in DPPH is reduced upon receiving a hydrogen atom from an antioxidant. The DPPH free radical scavenging effect expressed as percent inhibition of waxy pigmented and non-pigmented rice at various concentrations are shown in Table 2. The percentage of inhibition of the DPPH radical by the extracts of the waxy pigmented rice was observed to increase with increasing concentration which exhibited the highest percentage inhibition of 75.37%. However, the percentage of inhibition of the DPPH radical by the extracts of the waxy non-pigmented rice decreased with increasing concentration which is 6.72% in total phenolic concentration of 1.7 ug GAE g^{-1}.

*Copper reducing activity.*Copper reduction antioxidant capacity (CUPRAC) test is a variant of FRAP test using Cu instead of Fe. The assay is based on the reduction of Cu^{+2} to Cu^{+1} by the combined action of all antioxidants (reducing agents) in a sample. The Cu^{+1} then forms a yellow colored complex with neocuproine (2,9-dimethyl-1,10-phenanthroline) which absorbs at 450 nm. Therefore, the higher the absorbance at 450 nm, the higher the reduction of copper by the antioxidants; the higher the antioxidant capacity, the better the health beneficial quality of foods.

Copper, free and in phenanthroline complexes, has a lower redox potential than iron, so its reactions are more selective; sugars and citric acid, common interference with Ferric Reducing Ability of Plasma (FARP), are not oxidized in CUPRAC. At the same time, the low redox potential enhances redox cycling, so copper reduction may be an even more sensitive

indicator of potential pro-oxidant antioxidants. In the present study, the percentage of copper reducing activity of the waxy pigmented and non-pigmented rice extracts was observed to increase with increasing concentration of the total phenolic concentration (ug GAE g^{-1}) the highest percentage of 0.754% and 0.377 %, respectively (Table 3). However, the percentage of copper reducing activity was already not detected in the extracts of the waxy non-pigmented rice at concentration of 20 ug GAE mg^{-1}.

Consumption of waxy pigmented rice resulted in reducedartherosclerotic lesions (Ling*et al.,* 2001.), reduced oxidative stress and inflammatory effects (Xia*et al.,* 2003). Antioxidant activity in white rice hull had already been reported by Lee *et al.* (2003). Pigmented rice is composed of high content of phenolic compounds (Oki et al., 2002 and Clifford, 2000). They are distributed in the plant as secondary structure metabolite (Matinez-Valverde*et al.,* 2000). Various benefits of the phenolic compounds are known to have various effects in human including oxidative damage of lipid, low density lipoproteins inhibiting platelet aggregation (Daniel*et al.,*1999), and reduced coronary heart disease and cancer risk (Matinez-Valverde*et al.,* 2000). Fruits and vegetables are major dietary sources of phenolic compounds (Tian*et al.,*2004), however, waxy pigmented and non-pigmented rice have been found as an excellent source of the phenolic compounds. Waxy pigmented rice has 2.1% for amylose, 8.31% for crude protein, 2.67 % crude fat, 0.77 % crude fiber, 0.59 % crude ash, 7 mm for length, 1.8 mm for width, 16.57 g weight/1000grain, 2047 ug g^{-1} for total phenolic content, 12.54 % for DPPH and 0.138 % CUPRAC compared to waxy non-pigmented rice but the 86.69 % of starch and 11.7 % of moisture content was lower compared to waxy non-pigmented rice. So the waxy pigmented rice has higher TP, DPPH and CUPRAC than those in waxy pigmented rice.

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THE POTENTIAL USE OF ULTRAVIOLET-VISIBLE SPECTROSCOPY AND SOFT INDEPENDENT MODELLING OF CLASS ANALOGIES (SIMCA) FOR CLASSIFICATION OF INDONESIAN PALM CIVET COFFEE (KOPI LUWAK)

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ABSTRACT

In this study, the potential use of ultraviolet-visible (UV-Vis) spectroscopy and soft independent modelling of class analogies (SIMCA) for classification of Indonesian palm civet coffee (kopi luwak) was investigated. A number of 20 samples were used. The samples consisted of 10 samples of pure civet coffee without any adulteration (non-adulteration) and another 10 samples of mixture civet coffee adulterated with Arabica coffee (adulteration). All samples were extracted using distilled water and filtered. The spectral acquisition was performed with 10 mm of cuvette cell using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) in the range of 200-450 nm. The result showed that using principal component analysis (PCA) of moving average smoothing spectra, a clear separation of non-adulteration and adulteration samples could be obtained. The SIMCA classification method showed that it is possible to classify and separate the samples into two different classes (non-adulteration and adulteration samples) with accuracy, sensitivity and specificity was more than 90%. This result will open a development of a quick and reliable method based on UV-Vis spectra for civet coffee authentication in near future.

Keywords: UV-Vis spectroscopy, chemometrics, SIMCA, PCA, classification**1.**

INTRODUCTION

Coffee is one of the most important food commodities worldwide. Among all commodity traded in the world, coffee is number two after crude oil (Esquivel and Jiménez, 2012). There are two important species of coffee which has economic significance in the global coffee trade, species Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Another important type of coffee is Luwak coffee or Asian palm civet coffee or Kopi Luwak

(Indonesian words for coffee and palm civet) which is well known as the world's priciest and rarest coffee (Marcone, 2004). Luwak coffee is any coffee bean (Arabica or Robusta) which has been eaten and passed through the digestive tract of Asian palm civet (*Paradoxurus hermaphroditus*), which uses its keen senses to select only the best and ripest berries. As a result, its rarity as well as the coffee's exotic and unique production process ultimately accounts for its high selling price, approximately a hundred times higher than regular coffee (International Coffee Organization, http://www.ico.org/prices/pr-prices.pdf).

As one of the most profitable trading products, Luwak coffee has been a target for fraud trading by mixing Luwak coffee with other cheaper coffee. In order to protect the authenticity of Luwak coffee and protect consumer from Luwak coffee adulteration, it is very important to develop a robust and easy method for adulteration detection and quantification in Luwak coffee. Recently, food authentication is a major challenge that has become increasingly important due to the drive to guarantee the actual origin of a product and for determining whether it has been adulterated with contaminants or filled out with cheaper ingredients (Ashurst and Dennis, 1996; Singhal *et al.,* 1997).

At present, there is no internationally accepted method of verifying whether a bean is civet coffee or Luwak coffee. Traditionally, coffee aroma has been used to characterize coffee quality. Sensory panel evaluation is commonly used to assess the aroma profile of coffee. However, this technique has some limitations. For example, it is quite difficult to train the panel effectively in order to limit subjectivity of human response to odors and the variability between individuals (Shilbayeh and Iskandarani, 2004). Indonesia as one of important player in civet coffees production is now just starting to develop an advanced technology for coffee processing. It is including a search for a novel inspection system for civet coffees characterization. This technology is very important for coffee industry to protect high expensive civet coffees from any adulteration. In the previous study, Souto *et al.* (2010) reported the use of UV-Visible spectroscopy as an analytical method for the identification of adulterations in ground roasted coffees (due to the presence of husks and sticks). This analytical method is one of the most common and inexpensive techniques used in routine analysis and it will be compatible with situation in Indonesia for further technology development. Therefore, in this research, we attempt to use UV-Visible spectra combined with chemometrics methods (SIMCA/soft independent modelling of class analogy) to

establish a rapid and simple method for discrimination of Luwak coffee and its adulteration.

2. MATERIALS AND METHODS

2.1. Sample preparation

A number of 1 kg ground roasted Luwak Robusta coffee (Indonesian palm civet coffee) samples were collected directly from coffee farmers at Liwa, Lampung, Indonesia (Hasti coffee Lampung). Another 1 kg ground roasted Arabica coffee samples were also provided for making Luwak coffee adulteration. All coffee samples were grinded using home-coffee- grinder (Sayota). Since that particle sizes in coffee powder has significant influence to spectral analysis, it is important to use same particle size in coffee powder samples (Suhandy *et al.,* 2016). In this research we use particle size of 420 µm by sieving through a nest of U. S. standard sieves (Mesh number of 40) on a Meinzer II sieve shaker (CSC Scientific Company, Inc. USA) for 10 minutes. The experiments were performed at room temperature (around 27-29°C). In this research we prepared 20 samples of coffee samples which consist of two classes, class A and B. Class A has 10 samples of Luwak coffee without adulteration. Class B has 10 samples of Luwak coffee with adulteration (adulterated with Arabica coffee in the range of adulteration 10-50%).

An aqueous extraction procedure of the coffee samples was performed as described by Souto *et al.* (2015). First, 1.0 g of each sample was weighed and placed in a glass beaker. Then, adding 10 mL of distilled water at 90-98°C then mixed with magnetic stirring (Cimarec™ Stirrers, model S130810-33, Barnstead International, USA) at 350 rpm for 5 min. Then the samples were filtered using a 25 mm pore-sized quantitative filter paper coupled with an Erlenmeyer. After cooling process to room temperature (for 20 min), all extracts were then diluted in the proportion of 1:20 (mL: mL) with distilled water. UV-Vis-NIR spectra from the aqueous extracts were acquired using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA).

2.2. Instrumentation and spectra data acquisition

UV-Vis-NIR spectra in the range of 200-450 nm were acquired by using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) equipped with a quartz cell with optical path of 10 mm, and spectral resolution of 1 nm at 27-29°C. Before the

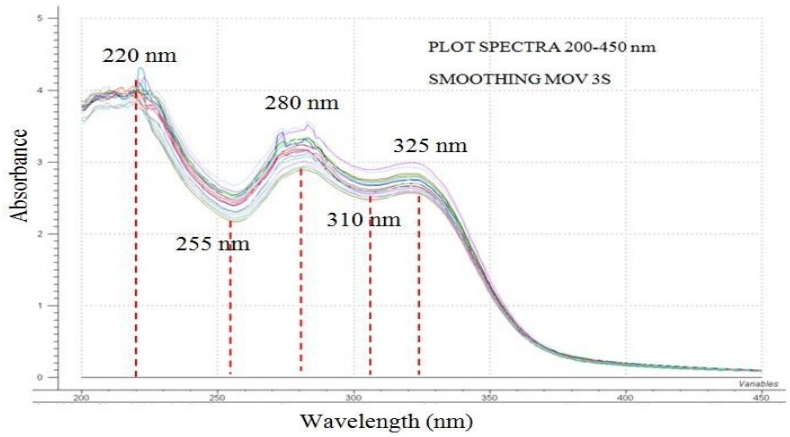
measurement step, blank (the same distilled water used in extraction process) was placed inside of the sample cell to adjust the 100% transmittance signal.

2.3. Data analysis

All recorded spectra data were transferred to computer via USB flash disk and then convert the spectra data from .csv extension into an excel data (.xls). All spectrum then smoothed using moving average algorithm with number of averaging is 3 segments. For classification, a SIMCA model was developed using PCA (principal component analysis) for each class. The calculation of smoothing spectra and developing SIMCA model were done using a multivariate analysis program The Unscrambler® version 9.8 (CAMO AS, Norway). The performance of classification result was evaluated using three parameters: accuracy, sensitivity and specificity (Lavine, 2009).

3. RESULTS AND DISCUSSION

Fig. 1 shows the result of spectral acquisition of 20 samples of Luwak coffee with and without adulteration in the range of 200-450 nm. It is clear that all spectral data (20 samples) are almost identical. It is very difficult to see any differences between samples Luwak coffee with and without adulteration. Here, we identify several peaks at 220 nm, 255 nm, 280 nm, 310 nm and 325 nm. The peak at 220 nm and 280 nm however is little bit noisy. The peak at
280 nm is close to absorbance of caffeine which is also reported by several previous investigators (Belay *et al.,* 2008; Clarke and Macrae, 1985).



Wavelength (nm)

Figure 1. The spectral data of 20 coffee samples acquired using UV-Visible spectrometer in the range of 200-450 nm

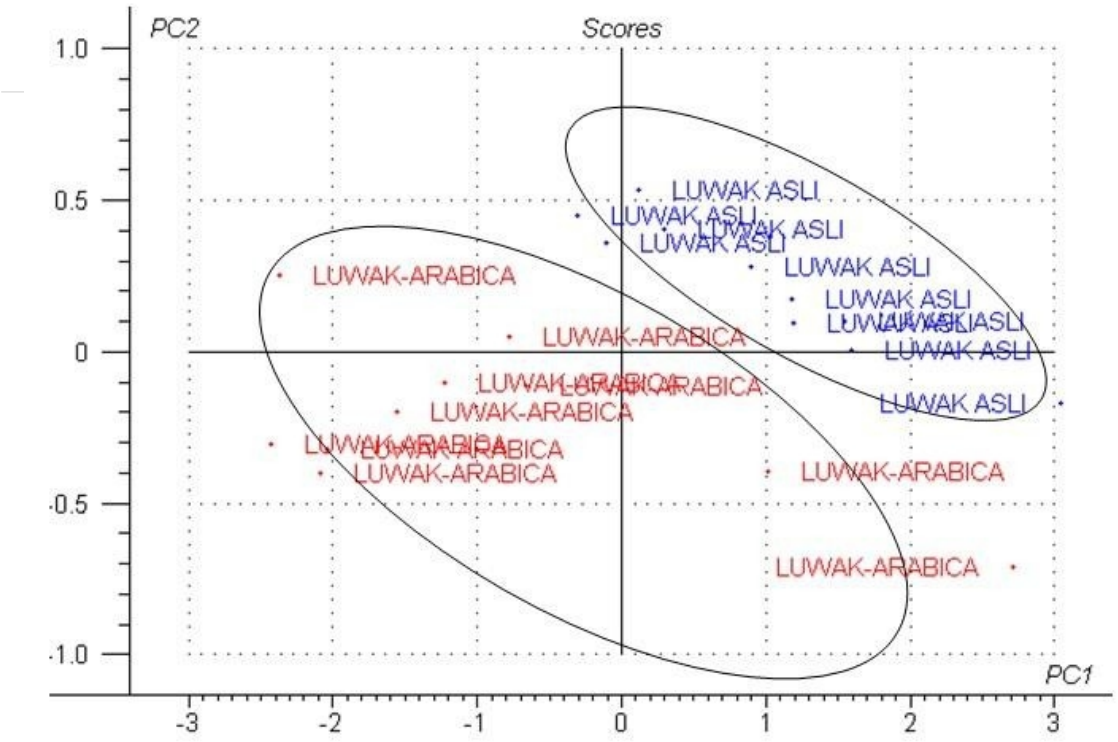


Figure 2. The scores of PC1 and PC2 of PCA using 20 samples.

Fig. 2 shows the result of global principal component analysis (PCA) of 20 samples (all samples). The figure shows the scores of PC1 and PC2 (in total explain 96% of sample variance). It can be seen that using PC1 and PC2, the samples can be divided roughly into two groups or two classes (luwak coffee without adulteration and luwak coffee with adulteration). This figure suggests us that the 20 samples can be separated into two different classes.

For this reason, in SIMCA analysis we perform PCA for two different classes, class A (luwak coffee without adulteration) and class B (luwak coffee with adulteration) with 10 samples for each class, respectively. Fig. 3 and 4 show the result of PCA for each class. In class A the total sample explained variance for PC1 and PC2 is 96% and in class B is 99%, respectively. It can be said both developed SIMCA model for class A and B are capable to explain and handle most variation contained in original coffee samples.

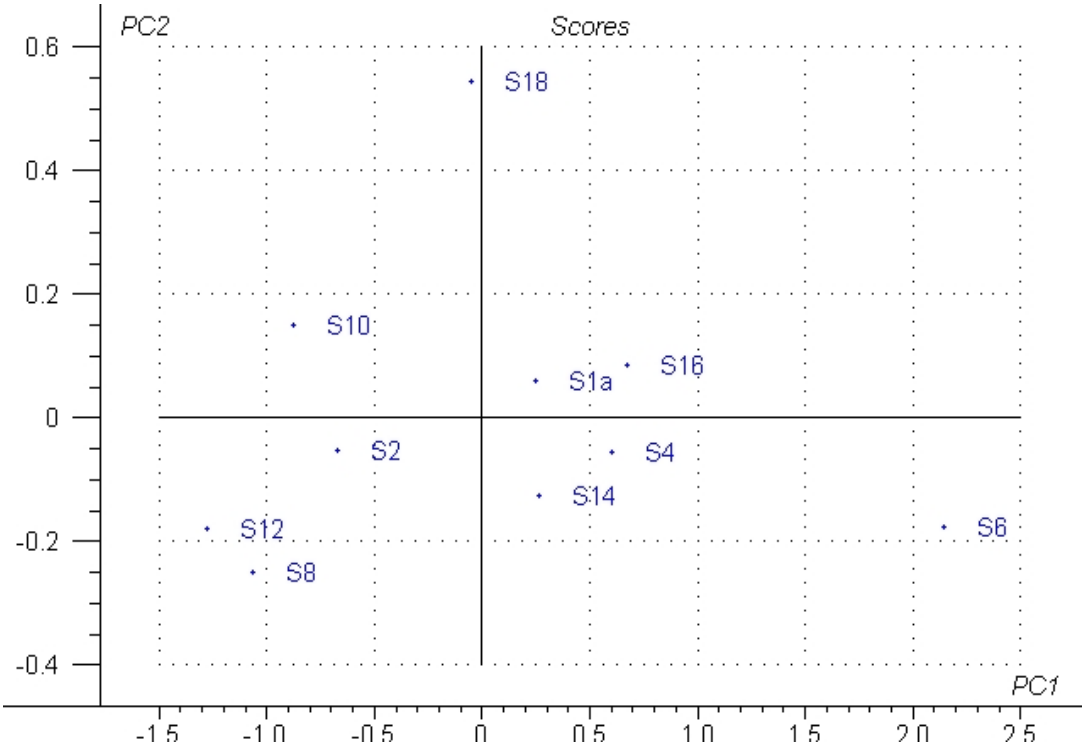


Figure 3. The result of PCA for samples in class A (luwak coffee without adulteration).

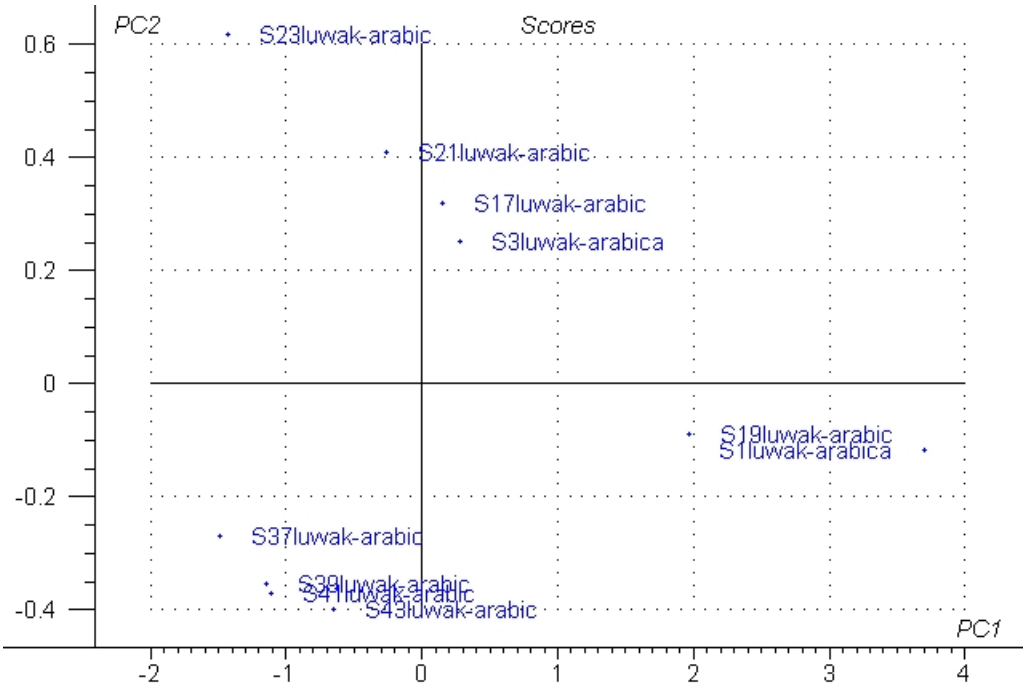


Figure 4. The result of PCA for samples in class B (luwak coffee with adulteration).

Sample	simcaaslil0samples	simcaCAMPURAN10samples
S1a	*	
S2	*	
S4	*	
S6	*	
S8	*	
S10	*	
S12	*	
S14	*	
S16	*	*
S18	*	
S1luwak-arabica		*
S3luwak-arabica		*
S17luwak-arabic		*
S19luwak-arabic		*
S21luwak-arabic		*
S23luwak-arabic		*
S37luwak-arabic		*
S39luwak-arabic		*
S41luwak-arabic		*
S43luwak-arabic		*

Figure 5. The result of classification using model SIMCA class A and B for 20 samples.

Fig. 5 shows the result of classification using developed SIMCA model for both class A and B at 10% significance level. It can be seen that sample number S16 is actually belong to class A (Luwak coffee without adulteration). However, the model classifies the sample S16 is belong to both class A and B. For other samples, the classification result is very satisfied where all samples successfully classify into proper class. For example, all samples from S1luwak-arabica until S43luwak-arabica are actually belong to class B (luwak coffee with adulteration) and the developed SIMCA model successfully classify all those samples into class B.

In order to calculate the performance of classification, a confusion matrix was created, with the accuracy, sensitivity and specificity of the coffee samples classifications using developed SIMCA models. It can be seen that SIMCA results were satisfactory (superior to
90%) for the accuracy, sensitivity and specificity rates.

Table 1. Confusion matrix with accuracy, sensitivity and specificity of the classification of coffee samples by SIMCA.

	Class A (assigned by model A)	Class B (assigned by model B)
Class A (actual)*	9	1
Class B (actual)	0	10
Accuracy (%)	95%	
Sensitivity (%)	91%	
Specificity (%)	100%	

This research demonstrates a potential use of UV-Visible spectroscopy along with chemometrics method to identify adulteration in Luwak coffees. The classification result was satisfactory with accuracy rate, sensitivity and specificity were 95%, 91% and 100%, respectively. This promising result has opened a possible application of using UV-Visible spectroscopy and chemometrics method to establish a rapid and acceptable method for classification of Indonesian Luwak coffee. This method may be applied to protect our Indonesian Luwak coffee from any adulteration using other cheaper coffees.

AKNOWLEDGEMENTS

We express our deepest appreciation to Hasti coffee Lampung, for providing coffee samples. We also gratefully acknowledge support of the Indonesian Ministry of Research, Technology and Higher Education (KEMENRISTEKDIKTI) via Penelitian Strategis Nasional (STRANAS) 2016 (Nomor: 419/UN26/8/LPPM/2016) and The University of Lampung, Indonesia. We thank to the Department of Agricultural Engineering, The University of Lampung for their permission to use their facilities for spectral data acquisition.

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DETECTION AND QUANTIFICATION OF ADULTERATION IN LUWAK COFFEE THROUGH ULTRAVIOLET-VISIBLE SPECTROSCOPY COMBINED WITH CHEMOMETRICS METHOD

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ABSTRACT

Luwak coffee is the most expensive coffee in the world and currently, the authentication of Luwak coffee has become very important due to the possible adulteration of Luwak coffee with non-Luwak coffee. In this research, we investigated the potential application of using ultraviolet-visible (UV-Vis) spectroscopy combined with chemometric techniques (partial least square/PLS1) for quantification of adulteration in Luwak coffee. The adulterant was Arabica coffee which was added into Luwak coffee with degree of adulteration in the range of 10-50%. A number of 30 samples were used. All samples were extracted with distilled water and filtered. The spectral acquisition was performed with 10 mm of cuvette cell using a UV - Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) in the range of 200-500 nm. PLS1 model correlates the actual and UV-Vis estimated values of adulterants (concentration of Arabica coffee in Luwak coffee) with coefficients of correlation (r) of 0.99 and 0.97 for calibration and validation, respectively. The low RMSECV values of 0.044 gram could be obtained. The method, therefore, is potential to be used as a rapid method for quantification of adulterant in Luwak coffee.

Keywords: Luwak coffee, chemometrics, PLS1 regression, calibration, UV-Vis spectroscopy

1. INTRODUCTION

Coffee is one of the most popular beverages in the world (Duarte *et al.,* 2005) with high consumption level in developed countries; 4 kg per capita in the US and 5 kg per capita in Europe (http://www.worldmapper.org/posters/worldmapper_1038_coffee_consumption_ver2.pdf). On other hand, coffee is mostly produced in several developing countries. Four countries account for more than half of the world's production: Brazil, Vietnam, Colombia and Indonesia. (http://www.worldmapper.org/posters/worldmapper_1037_coffee_production_ver5.pdf).

Luwak coffee is a name for Arabica or Robusta coffee which has been eaten by Asian palm civet (*Paradoxurus hermaphroditus*). The coffee bean which is eaten by Asian palm civet (*Paradoxurus hermaphroditus*) is the best and ripest berries. After several hours inside the civet animal, the best coffee berries passed through the digestive tract of civet animal resulted in a unique flavour of Luwak coffee. This kind of unique production is a reason why the production of Luwak coffee is very limited and worldwide it is approximately only 250-500 kg per year (http://www.most-expensive.coffee).

Luwak coffee is one of the most expensive and the rarest coffee in the world. Due to its commercial importance, detection of adulterated matters has been a constant concern in fraud verification, especially when it is difficult to percept adulterations with the naked eye in samples of Luwak roasted coffee ground. The inspection of adulteration materials becomes more difficult in samples of Luwak powder coffee. In Indonesia, Luwak coffee is adulterated with other cheaper non-Luwak coffee. Around 70% of Luwak coffee or civet coffee available at coffee stores and the internet (online store) is not 100% pure Luwak coffee and sometimes it does not contain anything of the genuine coffee (http://www.most-expensive.coffee).

In order to protect the authenticity of Luwak coffee, it is important to develop a simple method which can be used to detect and quantify the degree of adulteration. Recently, there is no internationally accepted method of verifying whether a bean is civet coffee or Luwak coffee. Traditionally, coffee aroma has been used to characterize coffee quality. Sensory panel evaluation is commonly used to assess the aroma profile of coffee. However, this technique has some limitations. For example, it is quite difficult to train the panel effectively in order to limit subjectivity of human response to flavour and the variability between individuals (Shilbayeh and Iskandarani, 2004). Human sensory method is also difficult to verify the authenticity of Luwak coffee when a small amount of adulterated materials such as cheaper non-Luwak coffee is added. Indonesia, as one of important player in Luwak coffee production, is now just starting to develop an advanced technology for coffee processing. It is including a search for a novel inspection system for Luwak coffees characterization.

This technology is very important for coffee industry to protect high expensive Luwak coffees from any adulteration. In the previous study, Souto *et al.* (2010) reported the use of UV-Visible spectroscopy as an analytical method for the identification of adulterations in ground roasted coffees (due to the presence of husks and sticks). This analytical method is one of the most common and inexpensive techniques used in routine analysis and it will be compatible with situation in Indonesia for further technology development. For this reason, in this research,

we attempt to use UV-Visible spectra combined with PLS regression method to detect and quantify content of adulterant in Luwak- Arabica blend coffee samples.

2. MATERIALS AND METHODS

2.1. Sample preparation

A number of 1 kg ground roasted Luwak coffee (Indonesian palm civet coffee) samples were collected directly from coffee farmers at Liwa, Lampung, Indonesia (Hasti coffee Lampung). Another 1 kg ground roasted Arabica coffee samples were also provided for making Luwak coffee adulteration. All coffee samples were ground using a home-coffee-grinder (Sayota). Since that particle sizes in coffee powder has significant influence to spectral analysis, it is important to use same particle size in coffee powder samples (Suhandy *et al.,* 2016). In this research we use particle size of 420 µm by sieving through a nest of U. S. standard sieves (mesh number of 40) on a Meinzer II sieve shaker (CSC Scientific Company, Inc. USA) for 10 minutes. The experiments were performed at room temperature (around 27-29°C). In this research we prepare 30 samples of Luwak-Arabica blend coffee samples which different content of adulteration. The adulteration content range is 0-50% by adding Arabica coffee into Luwak coffee samples.

The spectral acquisition of Luwak-Arabica blends coffee samples were done in solution samples. For this purpose, an aqueous extraction procedure of the coffee samples was performed as described by Souto *et al.* (2010). First, 1.0 g of each sample was weighed and placed in a glass beaker. Then, adding 10 mL of distilled water at 90-98°C then mixed with magnetic stirring (Cimarec™ Stirrers, model S130810-33, Barnstead International, USA) at 350 rpm for 5 min. Then the samples were filtered using a 25 mm pore-sized quantitative filter paper coupled with an erlenmeyer. After cooling process to room temperature (for 20 min), all extracts were then diluted in the proportion of 1:20 (mL: mL) with distilled water. UV-Vis-NIR spectra from the aqueous extracts were acquired using a UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA).

2.2. Spectral acquisition

The spectral data of coffee blend (Luwak-Arabica) were acquired using UV-Vis spectrometer (Genesys™ 10S UV-Vis, Thermo Scientific, USA) in the range of 200-450 nm. This spectrometer is a dual-beam spectrometer equipped with 5 cell sample holder and 1 cell for blank or reference holder. The wavelength accuracy is 1 nm with dual silicon photodiodes as detector and Xenon flash as illumination source.

The absorbance data of Luwak-Arabica coffee blend in solution samples were acquired in the range of 200-450 nm at room temperature. For this, we put 2 mL of solution samples into cuvettes. Before the sample measurements step, blank (the same distilled water used in extraction process) was placed inside reference cell holder to adjust the 100% transmittance signal.

2.3. PLS regression

The correlation between spectral data and content of adulteration (content of Arabica) was investigated using partial least squares (PLS) regression. The spectral data has many overlapped information. Some information is important and it has strong relation to the target response (content of adulteration). However, some information is not related to the target response. So, the general idea of PLS is to try to extract those information. PLS find several latent factors which account for most of the variation in the response. For this reason, the acronym PLS has also been taken to mean ‘‘projection to latent structure.’’ It should be noted, however, that the term ‘‘latent’’ does not have the same technical meaning in the context of PLS as it does for other multivariate techniques. In particular, PLS does not yield consistent estimates of what are called ‘‘latent variables’’ in formal structural equation modelling (Dykstra, 1983). PLS regression model has been used widely for multivariate data analysis including spectral data for qualitative and quantitative analysis from UV to terahertz region (Suhandy *et al.,* 2012; Shan *et al.,* 2014)

In this study, PLS regression model was developed using The Unscrambler® version 9.7 (CAMO, Oslo, Norway), statistical software for multivariate calibration. A student’s t-test was performed using Statistical Package for the Social Science (SPSS) version 11.0 for Windows in order to evaluate the significance level of the model.

3. RESULTS AND DISCUSSION

3.1. Spectral data of Luwak-Arabica coffee blend

In Fig. 1 we can see the original spectra of coffee blend (Luwak-Arabica) in the range of 200-450 nm. We notice the difference of absorbance intensity due to difference of content of adulterant (content of Arabica). However, the spectral difference is not consistent and it may be due to baseline differences. In order to remove the influence of baseline effect, we processed the spectra using smoothing and derivation algorithm (Savitzky-Golay 1st derivative with polynomial order 2 and number of segments 11) as shown in Fig. 2. Here we can see that it is not easy to see the spectral differences among the samples having different content of adulterant. So it is really necessary to use multivariate analysis to extract such kind of spectral information. From Fig. 1 and 2 we can see that there are several peaks which may

be related to the information of content of adulteration. The peak at 280 nm can be found both in original and processed spectra. This wavelength is close to the wavelength related to caffeine absorbance at 272 nm in the previous study (Belay *et al.,* 2008). The shift of caffeine absorbance peak (from 272 nm to 280 nm), it might be happen due to the presence of other component in coffee solution (not pure distilled water).

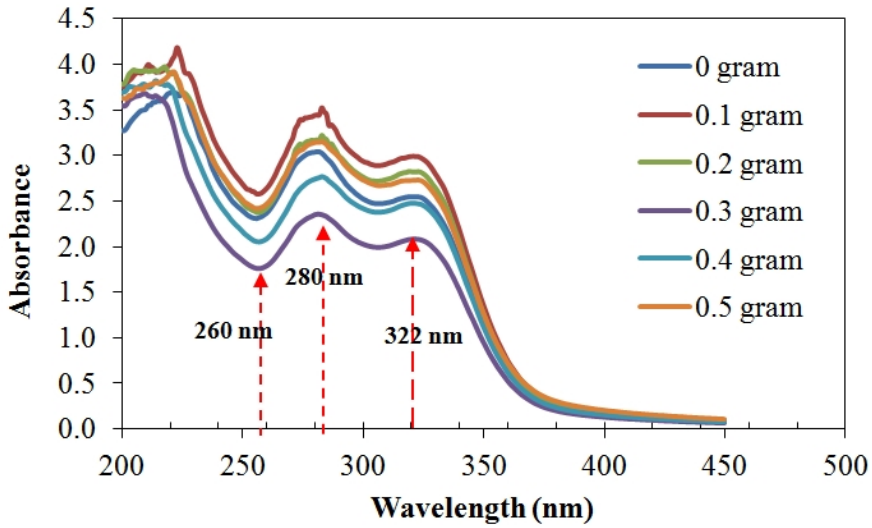

Figure 1. Original spectra of coffee blend (Luwak-Arabica) with different content of adulterant (Arabica) in the range of 200-450 nm

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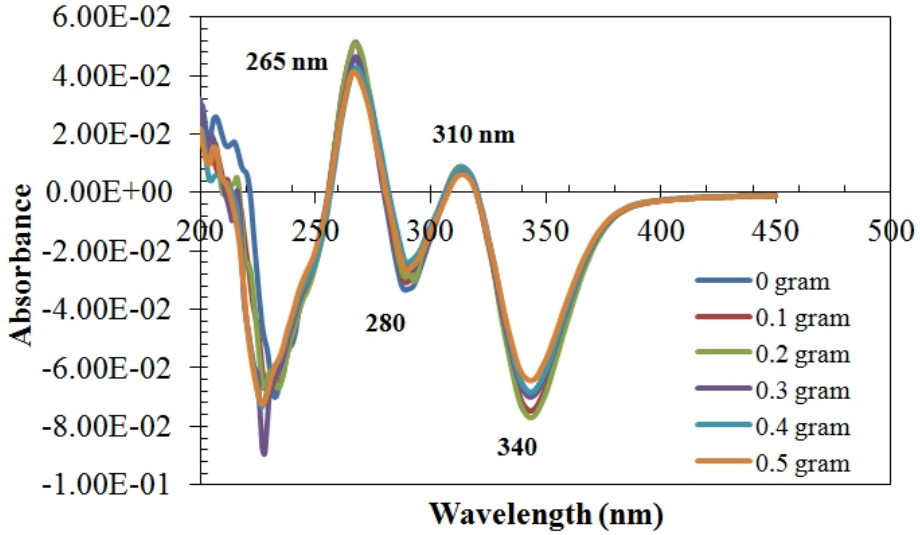

Figure 2. Processed spectra (smoothing +Savitzky-Golay derivation) of coffee blend (Luwak- Arabica) with different content of adulterant (Arabica) in the range of 200-450 nm.

3.2. Developing a PLS regression model for prediction content of adulteration

Here we perform a quantitative study for prediction content of adulteration in Luwak-Arabica coffee blend. For this purpose, we develop a PLS regression model using all wavelength in the range of 200-450 nm as predictor (*x* variables) and content of adulteration

or content of Arabica coffee added into Luwak-Arabica coffee blend as target response (*y* variable). Fig. 3 shows the result of PLS regression model in calibration step. It can be seen that there is a strong correlation between actual content of adulteration and predicted one using UV-Vis spectra with high coefficient of determination (R^2) = 0.99 and low RMSEC = 0.013884 gram. The developed PLS model was well validated as shown in Fig. 4. The cross-validation resulted in low RMSECV = 0.044242 gram and low bias = 0.003850 gram.

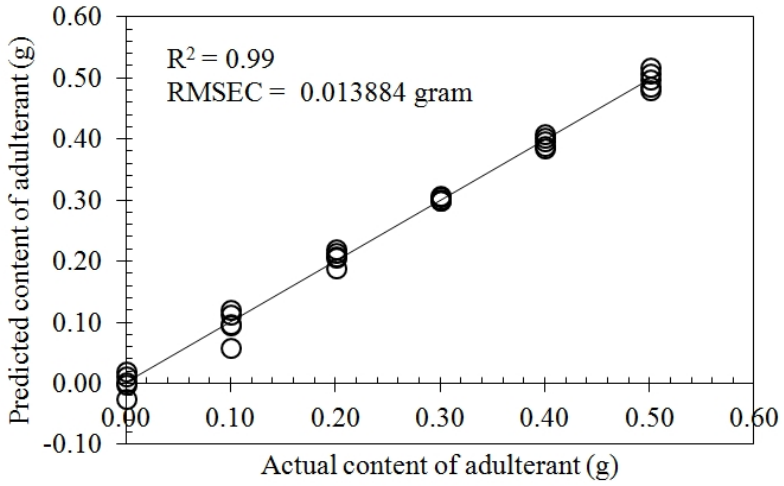

Figure 3. Scatter plot between actual and predicted content of adulteration in calibration step in the range 200-450 nm.

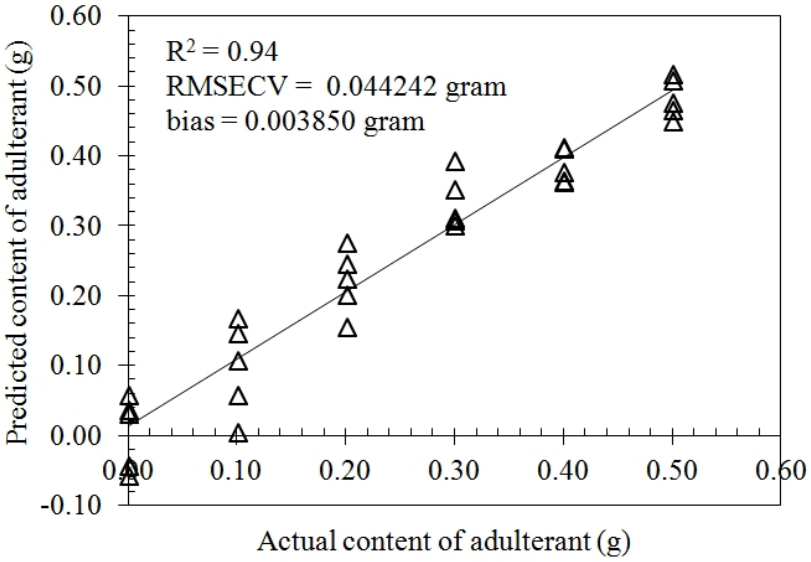

Figure 4. Scatter plot between actual and predicted content of adulteration in cross-validation step in the range 200-450 nm.

By a 95% confidence pair t-test, there were no significant differences between the actual content of adulteration and that predicted by UV-Visible spectroscopy. This result

showed that a calibration model for determination content of adulteration in Luwak-Arabica blend using UV-Visible spectroscopy could be well developed.

In order to understand the structure of the developed PLS model, we plot a relationship between the wavelength and regression coefficient as shown in Fig. 5. It can be noticed several wavelengths have significant value of regression coefficient. Those wavelengths are 275 nm, 300 nm, 342 nm and 378 nm. The wavelength at 275 nm is related to absorption of caffeine. The wavelength at 300 nm may be related to absorption of caffeic acid (Souto *et al.,* 2010).

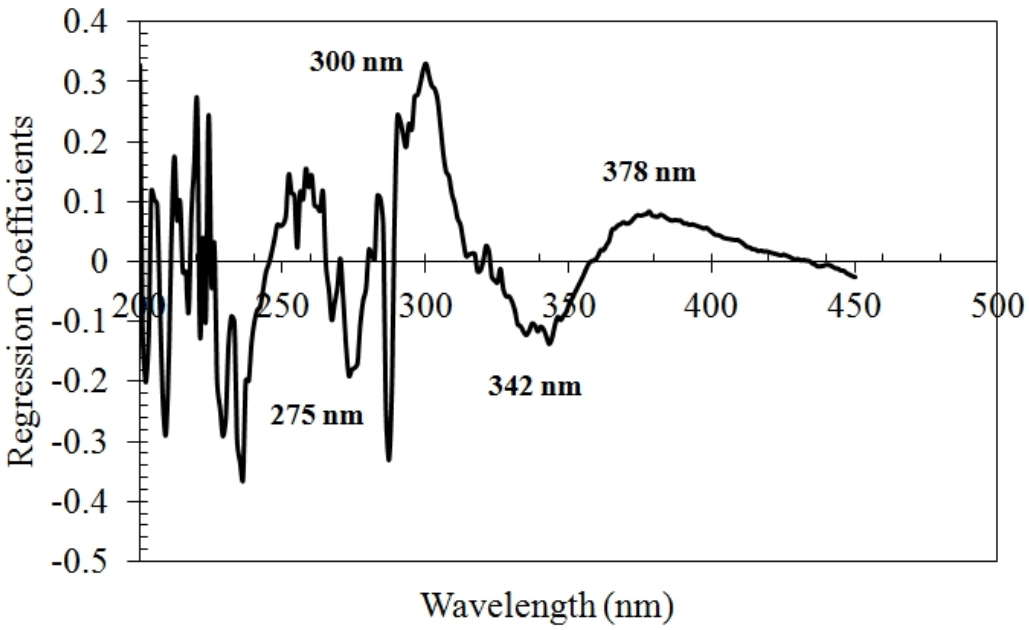

Figure 5. The regression coefficient versus wavelength plot of PLS model determination for prediction content of adulteration in Luwak-Arabica coffee blend.

This study has demonstrated the promising application of using PLS regression model for prediction the content of adulteration in Luwak-Arabica blend coffee samples. The developed PLS model resulted in a strong correlation between actual and predicted content of adulteration with R^2 = 0.99. The cross-validation resulted in low bias. By a 95% confidence pair t-test, there were no significant differences between the actual content of adulteration and that predicted by UV-Visible spectroscopy. This result may open a development of simple and fast method to detect and quantify the content of adulteration in Luwak-Arabica blend coffee samples.

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BIODIVERSITY OF BIRD SPECIES (Case Study: In KPHP Gedong Wani Desa Karang Rejo Kecamatan Jati Agung Lampung Selatan)

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ABSTRACT

KPHP Gedong Wani is a management system in production forest area by forestry goverment in Lampung Province, Indonesia. Gedong Wani area is also as one of the habitat for biodiversity of bird and this research is the first observation since 2011 - 2016.The research location in KPHP Gedong Wani (Study case in Karang Rejo Village, June 2016) was conducted to identify and analysis the diversity of bird species. The method had been used the concentrated in three different locations: (1) wetland area, (2) stand of *Hevea brasilliensis* area, and (3) stand of *Gmelina arborea area*. The result had been found 8 birds species of 6 families (N=118). The highest diversity (Shannon-Wiener index) was in stand of *Hevea brasilliensis* area (H'= 1.825), and the lowest was in wetland area (H'= 1,593).

Key words : Bird, diversity index, KPHP Gedong Wani, Lampung Province

INTRODUCTION

Ministry of Forestry decree No. SK 68 / Menhut-II / 2010 dated January 28, 2010 and the Decree of the Minister of Forestry No. SK 427 / Menhut-II / 2011 dated July 27, 2011 KPHP Gedong Wani area located in the region of South Lampung Regency and East Lampung Regency of Lampung Province of ± 30 243 ha. UPTD KPH Gedong Wani largely composed of two administrative area district, 11 districts and 39 village, so to that location for the presence of rare flora and fauna can say no more, but for the fauna wild unprotected still plenty for example cobras and birds herons and other birds that have not been identified type (Long Term Management Plan KPHP Gedong Wani, 2014). The location of the working area of UPTD KPHP Wani Gedong located on four registers Production Forest (KHP) is at KHP Way Katibung I Register 5, KHP Way Ketibung II Register 35, KHP Way Tibang Register 37 and KHP Gedong Wani Register 40. The administration area administration of

the UPTD KPH Gedong Wani most of his extensive serve as habitat for many species of wildlife.

The wildlife has a very important role for the sake of the balance of the ecosystem one of them is a bird that has a role as an agent of seed dispersers, pollinators of flowers and pest control (Alikodra, 1990), and can use enough space both horizontally and vertically (Wisnubudi, 2009) , Birds have high mobility and adaptability are spacious (Welty 1982; Goddess, 2005), both in conservation areas established by the government as it is in the nature reserves, wildlife sanctuaries and national parks, and outside conservation areas such as plantations , farmland, residential areas, plantations, forest production and cultivation area. Besides the bird is a species that has an important role as an indicator of biodiversity because of the bird has the properties that support, that life in all terrestrial habitats around the world, sensitive to environmental changes and taxonomy are clear and dissemination of geographic snya been quite unknown (Sujatnika *et al*. 1995). Diversity of bird species in one place is not the same as other places. Welty and Baptista in Parasasmita (2003) states that the presence of birds in a habitat very closely related to the physical environmental factors such as soil, water, temperature, sunlight and biological factors that include vegetation and other wildlife.

Biodiversity of bird spesies affected by the diversity of habitat types. The structure of vegetation and food availability in the habitat are the main factors that affect the diversity of species in a habitat (Tortosa 2000), As a component of bird habitats, phon can function as a cover (shelter from the weather and predators, nesting, play the rest and child care) (Setiawan 2006). so the habitat with more diverse vegetation variation will have a diversity of bird species higher in comparison to the habitat that has little vegetation. Bird conservation needs to be maintained by the conservation of bird species. Currently, the data and information on the diversity of bird species in the region is still limited. Therefore, research on the diversity of bird species in the KPH Gedong Wani important to provide accurate scientific information needs for conservation.

METHOD

The experiment was conducted in KPHP Gedong Wani village of Karang Rejo Agung Jati subdistrict South Lampung. KPHP Gedong Wani is located in two areas of government administration, namely South Lampung regency and East Lampung district consisting of 11 districts and 39 villages, while in geographical coordinates is located at 105 o 17 '40 "E up to

105 o 32' 35" E and 05 o 10 '00 "latitude up to 05 o 32' 30" latitude in June 2016 using concentrated (Bibby, Jones, and Marsden, 2000). Observations were made at three locations, namely observation: wetlands, vegetation rubber and Gmelina arborea vegetation can be seen (Figure 1 and Figure 2).

Observations was conducted in the morning at 6:00 a.m. to 08:00 pm. Bird identification refers to the "Book Bird Species Identification Field Guide in Sumatra, Java, Bali and Kalimantan" (MacKinnon, Philipps, and van Balen, 1998) as well as through interviews of people (Sugiyono, 2013). Diversity of bird species were analyzed using diversity index Shannon-Wienner (Odum, 1971; Fachrul 2007; Natarino, Dewi and Nurcahyani 2010; Main, Dewi, and Darmawan, 2011; Martin, Harianto and Goddess; Firdaus, Setiawan, and Lestari, 2012; Nababan, Setiawan and Nurcahyani, 2015; and Dewi Pamungkas, 2015; Pratiwi, Harianto and Dewi, 2015; Triyanah, Harianto and Goddess, 2015), with the following formula: H '= -Σ Pi ln (Pi), where Pi = (ni / N). Criteria values Shannon-Wiener diversity index (H ') are as follows: ≤ 1 (low diversity), 1 <<3 (diversity medium), and ≥ 3 (high diversity).

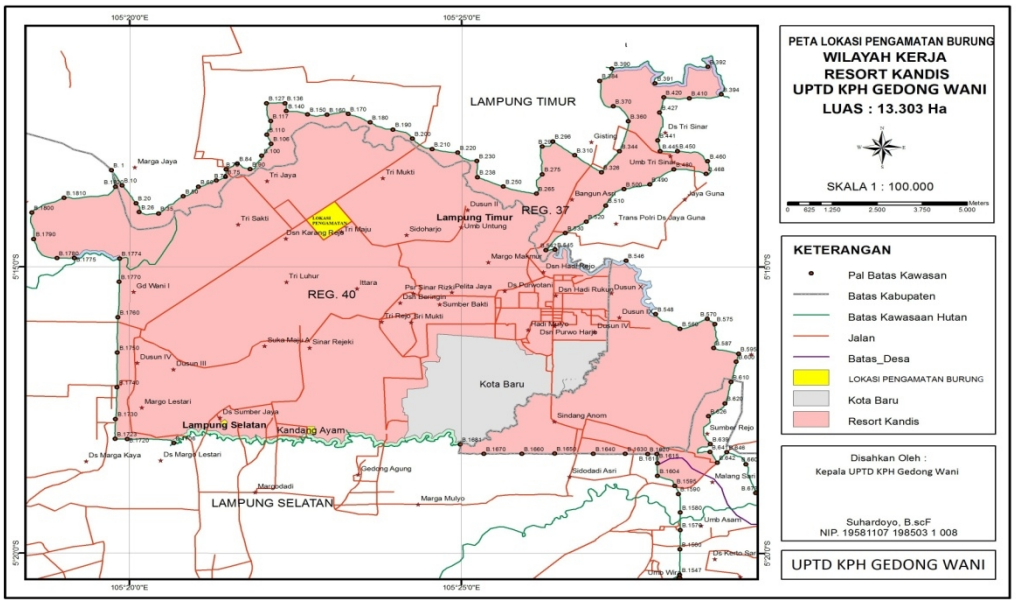

Figure 1. Map of the administrative work areas KPHP Gedong Wani scale of 1: 100,000 (Source: UPTD KPH Gedong Wani, 2016)

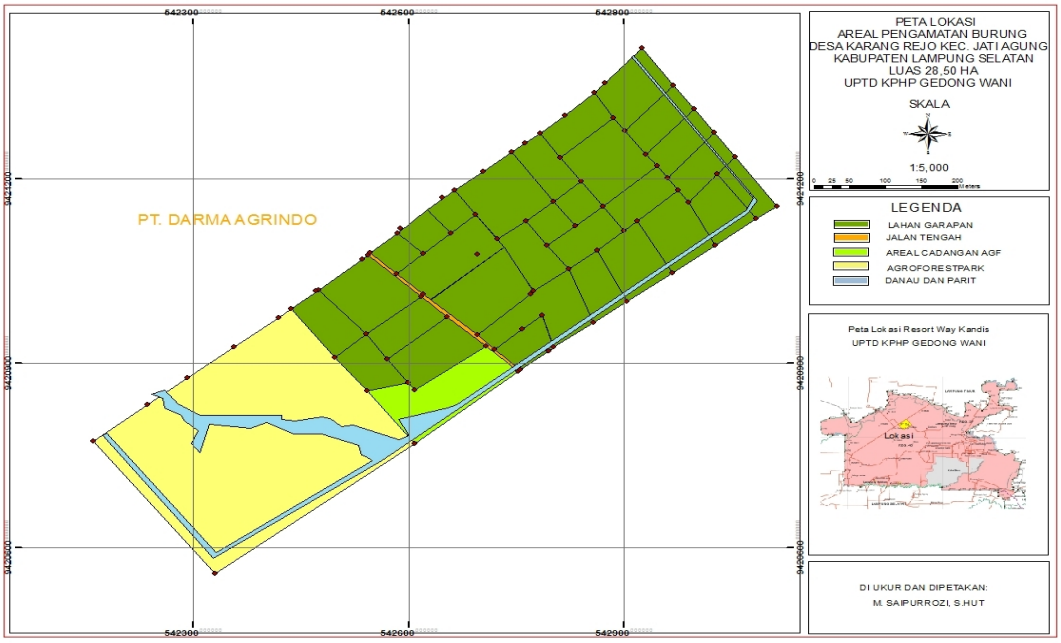

Figure 2. Location Karangrejo Bird in the village, district. Jati Agung scale of 1: 5,000 with Type 1 vegetation: Swamp, Vegetation 2: Wareng, Vegetation 3: Rubber (Source: Saipurozi, 2016).

RESULTS AND DISCCUSSION

Based on the research results, in three types of vegetation found 8 species of birds (N = 118 in number) are derived from 5 families (Table 1). The species most commonly found birds black-nest swiftlet (Collocalia maxima) (n = 29 mice). This bird is found in all locations with the largest number of observations on wetlands observation location. The location allows the mobility of birds is very good. According to MacKinnon et al (1998), this bird has a habit of flying and a circle in the air or fly low above the ground or water to catch small insects.

Most families that have species found are family Columbidae (Table 1) are doves (Streptopelia chinensis) and Turtledove (Geopelia striata). Turtledoves have characteristics Measuring + 30 cm and reddish-brown jambuan. Tail looks long with a thick white edges. Wing feathers are darker than body hair, there is a distinctive black stripes on the sides of the neck, a fine white speckled. Iris orange, black beak, red feet. Its habitat is usually in the woods, agorforest, plantations, settlements, and rice fields. The habits of living around the settlement and foraging on the ground. Often sit in pairs on the open road. Its distribution in Sumatra, Borneo, Java, and Bali (Paragraph 2011). Turtledove has a characteristic body is small (21cm) with the dominant color brown. On the back of the neck and side lines are thin,

gray face. Its habitat is in the forests, plantations (palm / rubber), agroforest, settlement and is generally found in the lowlands to an altitude of 900 m. Have a habit in pairs or in small groups, eating on the ground and often voiced especially in the daytime. Its distribution in Sumatra, Borneo, Java, and Bali (Paragraph 2011).

Table 1. Species of birds found in Gedong KPHP Wani Month June 2016

No	Type Nama	Scientific Name	Famili	Habitat			Amount
				A	B	C	
1	Cekakak Sungai	*Todirhamplus clhoris*	*Alcedinidae*	5	2	2	9
2	Cekakak belukar	*Halycon smyrnensis*	*Alcedinidae*	4	2	2	8
3	Cucak kutilang	*Pycnonotus aurigaster*	*Pynonotidae*	2	7	12	21
4	Bentet Kelabu	*Lanius schach*	*Laniidae*	0	9	5	14
5	Perkutut	*Geopelia striata*	*Columbidae*	0	9	8	17
6	Bondol Haji	*Lonchura maja*	*Ploceidae*	5	0	0	5
7	Tekukur	*Streptopelia chinensis*	*Columbidae*	2	7	6	15
8	Walet sarang hitam	*Collocalia maxima*	*Apodidae*	12	8	9	29

Information :
A = type of vegetation 1 swamp
B = type of vegetation 2 rubber (Hevea brasilliensis)
C = type of vegetation 3 Wareng (Gmelina arborea)

Collared kingfisher (*Todirhamplus clhoris*) included in the family Alcedinidae. This bird has a characteristic size of ± 24 cm, blue and white. Crown, wings, back and tail sparkling turquoise light and no black stripe passing through the eye. Collar and white lower body clean. Iris brown, dark gray upper half, the bottom half of a paler, gray legs. Its habitat is in the forests, agroforestry, plantations and settlements and have a habit of hunting large prey, slam-rocking first on a perch before eating. Its distribution in Sumatra, Kalimantan, Java and Bali (Paragraph 2011).

Cucak kutilang is a bird that belongs to the family Picnonotidae. This bird species is cucak-cucakan birds that eat fruits although also feed on insects. Bird species confidently with bustling chirp tend to live in trees. Cucak finch bird head a black cap with tunggir whitish yellow and orange upside-down, chin and upper kepa black. Collar, tunggir, chest and white belly.

Bentet Kelabu (*Lanius schach*) included in the family Laniidae. This bird has a characteristic size of ± 19 cm, red-backed brick. Part obviously thicker, shorter tail, and eyes bigger than Bentet Brown. Gray crown and nape; back, wings, and tail chestnut brown with

fine black lines, black eye stripe width, the lower body white, brown striped vague on the side of the body (male). Iris brown, blue-tipped beak black, gray legs. His voice was husky, grating babble, like the sound of Bentet Brown. Habitat in forests, plantations and open land to a height of 900 m above sea level. Has a habit of hunting insects from perches prominently on the edge of the forest. Its distribution in Sumatra, Kalimantan, Java and Bali (Paragraph 2011).

Bondol Hajj (*Lonchura maja*) belonging to the family Ploceidae. Has the characteristics of small (11 cm), brown, white-headed. In the juvenile bird brown upper body, lower body and face deep yellow. Similar Lonchura ferruginosa. The difference: more brown, the entire head and throat white. Iris brown, half-abukebiruan gray, pale blue feet. Spreading in Peninsular Malaysia, Sumatra, Java, Bali and Sulawesi, Thailand and southern Vietnam. In the introduction in Japan (Osaka and Okinawa). This bird is found, visit the swamp and marsh reeds up to a height of 1,500 meters above sea level. Forming large groups during the rice harvest, but spread out in pairs during the breeding season. Behavior generally as bondo (sparrow) Other (Kutilang Indonesia Foundation, 2016).

Swiftlet (*Collocalia maxima*) belonging to the family Apodidae. Has characteristics rather small size (13cm), blackish brown. Tunggir graded, from grayish be the same color as the back. Difficult to distinguish edible-nest swiftlet, but look more plump and rather straight truncated tail. Hairy legs. Iris brown, beak and black legs. Throughout the Greater Sunda. Most breeding near the coast of Sumatra and the islands around it. Swallow is most common in the limestone mountains in Borneo. Java is not common in small islands and coastal areas, but common in limestone mountains. Eating small insects caught when fly.

Make a nest of saliva white feather blend, embedded in limestone caves (called "black nests"). Taken for sale, but not as expensive as a white nest, because it requires a lot of time to remove the hair and tempayaknya. Rattling noises for ekholokasi. Laying two grains are elongated white. Nested on a seasonal basis, depending on the place to be (Kutilang Indonesia Foundation, 2016). Of the three locations of observation points obtained by the number of different species of birds. At the location of one type of vegetation found six species of birds with the number of individuals 30 individuals, in stands of vegetation type 2 Wareng found seven species of birds with tail number 44, in stands of gum damar agroforests found seven species of birds with the number of individuals 44 tails.

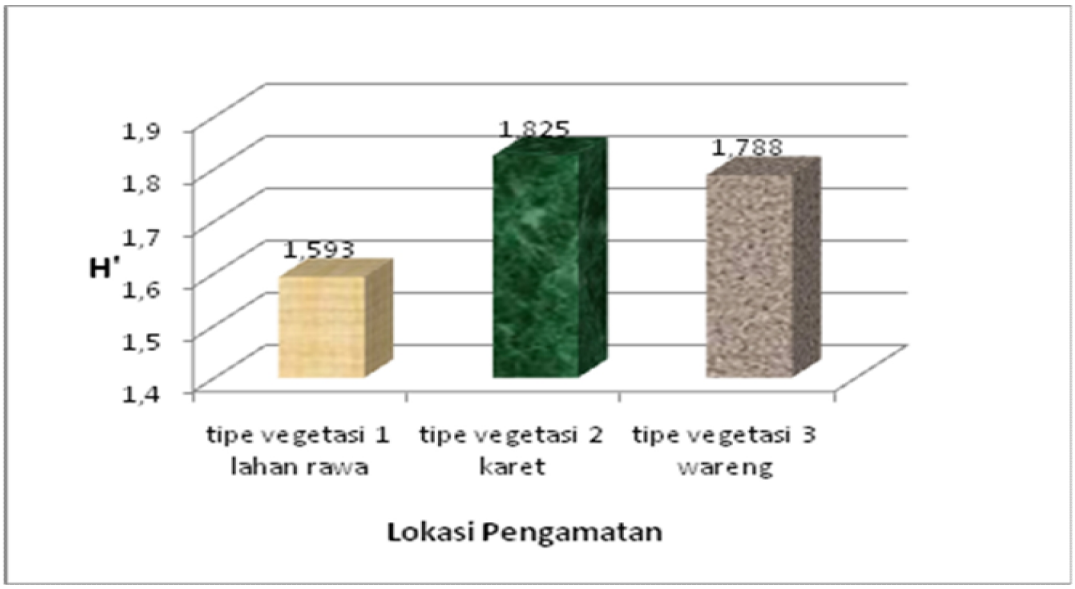

Figure 8. Histogram of species diversity index (H ') birds at three locations observation KPHP Gedong Wani village of Karang Rejo Agung Jati District of South Lampung

The highest diversity index values found in the location of vegetation rubber (H '= 1.825) with the criteria to have moderate levels of diversity (1 <H'≤3). Swamp vegetation observation sites (H '= 1.372), and the location of vegetation Wareng (H' = 1.788) also have a level of diversity that are (1 <H'≤3). The level of diversity that is showing that the site is still used as a residence, feeding and breeding ground for bird species. This is because these locations are supported by the vegetation is quite varied as feed for the birds, in contrast to locations with lower diversity. The loss of vegetation caused by the loss of a food source for birds, so that the timber cutting has a low bird diversity. Diversity of bird species associated with a balance in the community. If the value of diversity is high, then the balance of the community is also high. However, if the value does not necessarily indicate a high balance of species diversity in the community is high (Purnomo, Jamaksari, Revive, Pradityo, and Syafrudin, 2009).

In the study of birds by Utama, Dewi, and Darmawan (2011) found 43 species of birds with a total of 4101 individuals from 23 families. According Handari, Dewi, and Darmawan (2011) found as many as 29 species consisting of 14 families and individuals overall total 2642 individuals. According Rohadi, Dewi, and Darmawan (2011) at the University of Lampung swamp there are 17 species of birds from 11 families with a total of 1005. The results of individual birds in Pulau Anak Krakatau Islands Nature Reserve Region, there are

27 species of birds from 18 families and overall a total of 908 individuals (Martin, Harianto, and Dewi, 2012). According Natarino, Dewi, and Nurcahyani (2010) bird species found in the forest park management area of Sustainable SHK Wan Abdul Rachman total of 57 species were included in 23 families.

The number of birds found in the study site in KPHP Gedong Wani less compared to other bird research that has been done. This is due to the relatively short time that research so that researchers found little type and number of birds. In addition to birds, the research sites in KPHP Gedong Wani also found several species of other types. Here is a diagram that showed the presence of other types of species found in KPHP Gedong Wani obtained through interviews with the surrounding communities and employees / employee who works in KPHP Gedong Wani.

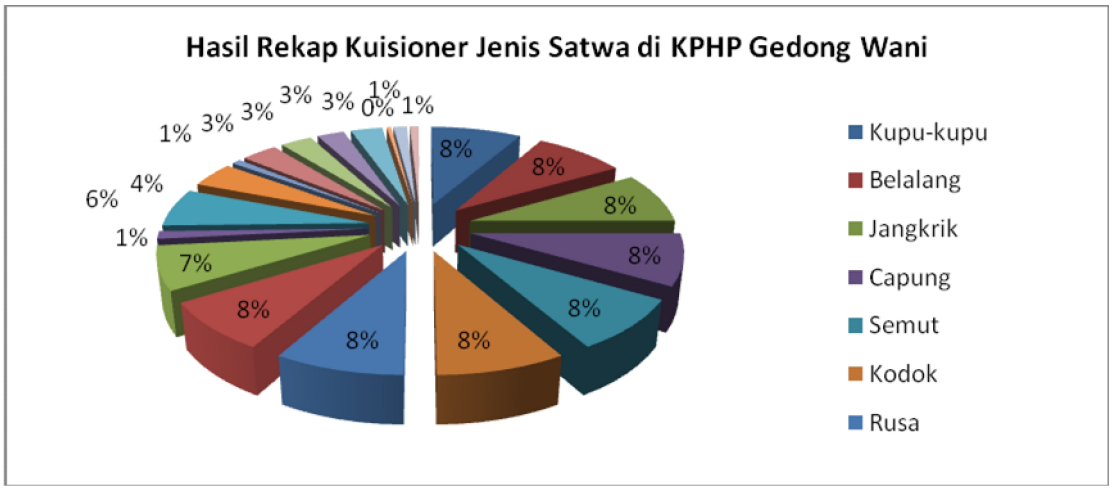

Figure 8. The species found in Gedong KPHP Wani

Another type species found in KPHP Gedong Wani than birds, insects such as butterflies, grasshoppers, crickets, dragonflies, ants and there are mammals such as chickens, cows, goats, and deer. There is also a frog. Kinds of insects found, can be used as food for birds. Most of these insects are found around the swamp and also in agroforestry KPHP Gedong Wani. The existence of these insects affect the availability of birds on the land.

Birds can be an indicator of the presence of the ecosystem. In KPHP Gedong Wani itself there agroforestry which is a pilot area of food security where there are rice under Gmelina stands. There are also plants fruits in the vicinity, such as longan, durian, papaya. Some plants such fruits into the food security program, because basically the principle of food security programs are the needs of the community when their staple food such as rice

can not be fulfilled due to climate change which causes the rice plant can not grow every season. Birds play a role here as well to spread the seeds of plants of the fruit. Besides the fruit trees are also an ingredient meal for the birds.

Based on the results of research in KPHP Gedong Wani in 2016 there were seven species of bird by the number of people 29 who came from 6 families. Bird species that most of the black-nest swiftlet (Collocalia maxima). The highest diversity index values found in the location of vegetation rubber (H '= 1.825), while the lowest level of diversity that is in the swamp vegetation (H' = 1.372).

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FATTENING OF BEEF CATTLE WITH NO GRASS: "EFFECT OF DIETARY ENERGY TO PROTEIN RATIO ON BEEF CATTLE FATTENING"

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ABSTRACT

An experiment was conducted to evaluate the effect of dietary energy to protein ratio in order to increase production and productivity of beef cattle. Particularly, during dry season when there was not enough grass as the main feed for ruminant, so complete feed (CF) ration was formulated without grass anymore, and it was replaced by using agricultural and/or agro-industrial by-product. Twenty tail of Simmental-cross animal with an average body weight 372.15 $\pm$ 26.64 kg were used in this experiment, were treated with complete feed of 11%, 12%, 13%, and 14% crude protein on iso-energy of 62% TDN (total digestible nutrients). As a dietary controlwas used farmer ration. *In vitro* technique was conducted to evaluate the fermentability of complete feed on the protein digestibility, and NH3 and VFA's rumen production. *In vivo* experiment was conducted for 100 days, based on completely randomized design. Data were calculated using analysis of variance. Compare to control diet with average daily gain (ADG) of 0.96 kg/h/d, increasing of dietary crude protein on iso-energy affected on increasing of ADG of the animal ranging 1.28-1.54 kg/h/d. Feed convertion ratio was decreased means that feed effiency utilization significantly increased. This results was supported by the *in vitro* experiment related with their ability to produce NH3 and VFA's rumen for maximum rumen microbial protein bio-synthesis.

Keywords: Beef, grass, protein

INTRODUCTION

Feed availability as quality, quantity, or continuity is important factor to support development of animal production system (Santoso and Hariadi, 2009). Further they stated in the tropical country such as Indonesia, grass production is much during wet season but very limited in dry season. Development feedstuff from agricultural land and crops by product is one of way to solve the problem. Central Java, one of the second largest cattle producers in Indonesia has the complex problem to provide feed availability especially during dry season. At present, In Central Java, many of the arable land would convert to housing and industry. This condition indicates the development of complete feed by utilization of agricultural land

and crop estate by products is needed to develop the cattle in the province. In the future, Yan et al., (2007) reported feeding technology with mitigation strategies to reduce N excretion in beef cattle is also needed. CF is the combination of concentrate and roughage in one diet. Utilization of agricultural and crop estate is one of way to provide feed for developing cattle in Central Java. Tamminga (1996) stated that every ruminant need carbohydrates, proteins, and lipids to maintain microbial rumen and produce products (meat, milk). Complete feed technology were developed use agricultural land and crop estate by products such as rice straw, cotton seed meal, coconut pod (Sunarso et al., 2012).

MATERIALS AND METHODS

Experiment 1. *In vitro* evaluation of complete feed

This experiment was aimed to evaluate ruminal fermentation *in vitro* of complete feed (CF) ration of iso-energy ration (62% TDN), and different level of crude protein (11%; 125; 13%; 14% CP). Table 1 showing the composition of complete feed ration.Crude protein digestibility, total protein production, rumen ammonia concentration, and total VFA'sconcentration were determined (Satter and Slyter 1974; Sutardi *et al.,* 1983). Feedstuffs and complete feed ration were analyzed according the procedure of AOAC (2000). Four complete ration were formulated with no grass anymore by using of agricultural waste and agro-industrial by product. Completely randomized design (CRD) were used in this experiment, and the differences among treatment then be avaluated using Duncan's multiple range test.

Experiment 2. *In vivo* evaluation of complete feed

Experiment 2 was aimed to evaluate the effect of iso energy ration and level of protein of complete feed (CF) as conducted in experiment 1 on the performance of beef cattle. Twenty male of Simmental Grade cattle with average body weight 372.15 $\pm$ 26.64 kg were arranged in CRD for treated with 5 ration and 4 replications. Ration consist of control diet as the farmer usually fed tothe animal (rice straw and concentrate), and 4 complete feed ration of iso-energy (62% TDN), and 4 level of CP (11%; 12%; 13%; 14%). All of the animal were fed 100 days, data collected such as follows: dry matter intake (DMI), average daily gain (ADG), feed convertion ratio (FCR).

RESULTS AND DISCUSSION

Experiment 1.

Results of Experiment 1 was shown in Table2.

Table 2 showing thatthere were different effect (p,0.05) of different experimental diet on the CP digestibility. This may caused the differentiation between the farmer ration (T_0) with CF ration. There were no differences among CF ration T_1, T_2, T_3, and T_4. No different effect were shown in total protein production *invitro*, total VFA's production andNH3 production *in vitro* in the rumen. The ration were formulated on the basis of iso energy, so, the production of total VFA's rumen were relatively similar among treatment. Although there were different level of crude protein, in fact that CP ration relatively higher than 10% as recommended by some experiments which showing that the ration had capability to produce of rumen ammonia concentration for maximum microbial protein synthesis (Satter and Roffler; Chalupa; Sutardi). In fact, that both of total VFA's and NH_3 were exactly enough for microbial protein synthesis as shown by the total protein production which come from dietary protein and microbial protein. The higher of the total protein produce the possibility of the protein synthesis woud be increased which would be reflected by the average daily gain of the animal.

Experiment 2.

Performance of beef cattle during 100 days feedingwith experimental complete feed ration which conducting in Experiment 2 was shown in Table3.

Table 3 showing that after 100 days of feeding there were no different results on the DMI of the animal ($p>0.05$) although, the animal treated with farmer ration consumed DMI higher than complete feed ration. This, indicated that both of farmer ration and CF ration possibly had similar taste and palatibility, so, DMI of all the animal relatively almost the same. Because of CF ration tend increased in CP content then CP consumption may increased both in quantity and quality, and since the protein digestibility (Table 2) were not affected by CF ration, it could be concluded that this protein was available for protein biosynthesis which was reflected on the increasing of ADG which significantly increased ($p<0.05$). In relation with DMI among treatment were similar quantitatively, so, feed convertion ratio as calculated by the total DMI kg/h/d) divided with ADG (kg/h/d) significantly decreased means that feed efficiency utilization increased. Increasing of protein level in the CF ration affected on the increasing body weight gain of the experimental animals, but at the level of 12.28% CP the average daily gain goes down as reflected by $Y = -0.1155\ X^2 + 0.757\ X + 0.356$.

Feeding complete feed ration showing similar DMI compare to that the farmer usually used. There were no different effect or negative effect in term of protein digestibility, total VFA's production *in vitro,* ammonia rumen production *in vitro,* total protein production, and

DMI. Compare to that the farmer ration, ADG of the experimental animal increased related with the increasing CP within ration, decreased their FCR or increased their feed efficiency utilization. Feeding with CF rationwith no grass anymore prospective to be developed particularly at the dry season when not enough grass availability.Because of the best performance of the experimental animals, it would be suggested that CF ration formulated at the 62% TDN and 12% CP

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Table 1. Complete feed (CF) composition of experimental diet

Feedstuff	T1	T2	T3	T4
	% DM			
Ammoniated rice straw	40.0	40.0	40.0	40.0
Yellow corn	10.0	10.0	10.0	10.0
Cassava	30.0	27.0	21.0	20.0
Rice bran	6.7	6.2	9.3	5.8
Kapok seed meal	1.0	3.0	4.5	8.0
Coffee hull	1.5	1.0	1.0	1.0
Palm oil	0.1	0.1	0.1	0.1
Copra meal	10.0	12.0	13.4	14.4
Urea	0.5	0.5	0.5	0.5
Mineral mix	0.1	0.1	0.1	0.1
Salt	0.1	0.1	0.1	0.1
Total	**100.0**	**100.0**	**100.0**	**100.0**
Nutrient Composition				
Crude Protein	11.54	12.01	12.95	13.61
Ether extract	2.67	2.57	3.29	3.23
Crude Fiber	24.41	26.67	23.21	25.63
Organik matter	89.84	90.28	89.47	90.54
Ash	10.16	9.72	10.53	9.46
Nitrogen free extract	51.22	49.03	50.02	48.07
TDN	62.81	60.90	64.59	62.67

Table 2. Digestibility and fermentability of experimental complete feed ration

Item	Treatments				
	T0	T1	T2	T3	T4
CP Digestibility, %	58.31^{b}	65.35^{a}	65.60^{a}	65.93^{a}	64.52^{a}
Total protein, mg/g	103.91	102.94	123.26	128.81	117.33
Total VFA's, mM	80.00	110.00	108.33	100.00	100.00
$NH_{3,\ mM}$	6.60	6.05	5.23	4.68	5.50

T_0: Farmer ration; T_1: T_2; T_3; T_4 (Iso TDN ration62%; and 11; 12; 13; 14% CP)

Table 3. Performance of beef cattle fed by experimental complete feed ration

Item	Treatments				
	T0	T1	T2	T3	T4
DMI, kg/h/d	11.77	10.02	11.52	11.14	9.40
DMI, % BW	2.83	2.18	2.54	2.42	2.11
ADG, kg/h/d	0.96^{a}	1.50^{b}	1.54^{b}	1.50^{b}	1.28^{b}
FCR	12.81^{a}	6.61^{b}	7.49^{b}	7.40^{b}	7.34^{b}

T_0: Farmer ration; T_1: T_2; T_3; T_4 (iso TDN ration62%; and 11; 12; 13; 14% CP)

EFFECTS OF THIDIAZURON AND BENZYLADENINE ON FORMATION OF SHOOTS AND EMBRYOGENIC NODULESIN BANANA (*Musa* spp.) TISSUE CULTURE

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ABSTRACT

A Study was conducted to investigate the effect of thydiazuron (TDZ) and benzyladenine (BA) on shoot and embryogenic nodule formation in banana tissue culture. The study consisted of two experiments. The first experiment was conducted to study response of banana cv Kepok Kuning to thidiazurona (TDZ) and benzyladenine (BA) in vitro. Shoot tips were used as explants and cultured on MS medium containing MS salts, 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glyciene, 30 g/l sucrose, combinations of TDZ (0.005, 0.01, 0.05, and 0.1 mg/l) and BA (0 and 2 mg/l) as treatments. The medium was solidified with 8 g/l agar. Some cultures formed embryogenic nodules, and these structures were then cultured on MS medium supplemented with 0.1 mg/l TDZ or 0.1 mg/l TDZ + 2 mg/l BA as treatments. The second experiment aimed to investigate response of banana (cv. Raja Bulu) embryogenic nodules to TDZ and BA as previously described in the first experiment. The medium was as previously described for the first experiment. Results of the first experiment revealed that BA did not significantly affected shoot and shoot bud. The highest number of shoots was attained at 0.05 mg/l TDZ (2.6 shoots per explant). Treatment 0.1 mg/l TDZ and 0.1 mg/l TDZ + 2 mg/l BA resulted in formation of embryogenic nodules. Results of the second experiment showed that increased in TDZ concentrations did not significantly affect embryogenic nodule proliferation. In contrast, addition of 2 mg/l BA significantly decreased number of embryogenic nodules, from 12.5 to 6.2 embryogenic nodules per explant.For propagation purpose, cv. Kepok Kuning required 0.005 mg/l TDZ for shoot and shoot bud multiplication and 0.1 mg/l TDZ for embryogenic nodule proliferation and cv. Raja Bulu required 0.005 mg/l TDZ for embryogenic nodule proliferation.

Key Words: Benzyladenine, Kepok Kuning, Raja Bulu, shoot tips, thidiazuron,

INTRODUCTION

Banana (*Musa* spp.),which belongs to the family of Musaceae, is one of the most important sources of food for human beings after wheat, rice, and corn (Remakanthan et al., 2014). Indonesia has been recorded as the sixth largest producers of banana fruit, producing 6.19 million MT, with India being the largest, with production of 26.5 million MT, followed by (in million MT), China 10.6, The Philippine 9.2, Ecuador 7.0, and Brazil 6.9 (Indian Horticulture Database, 2014). Indonesian production of banana has been mostly for domestic market, which is due to relatively low quality of products and their unreliable continuity. To supply the global market, this problem should be solved, among other things, by improving crop production practices and genetic quality of plants.

Most of banana production practices in Indonesia use suckers as planting materials, which are not reliable because they are in variable quality and difficult to supply in large number and in uniform size. One way to cope with the problem is by applying plant tissue culture technology because it could be used to produceuniform planting materials in a large number in a relatively short time.In addition, plant tissue culture could be used to support plant breeding through somaclonal variation, mutation, and genetic engineering. This technology is especially essential for commercial banana plants, since they are seedless and vegetatively propagated.

Plant tissue culture through axillary branching has been widely used to clonally propagate banana plants.Clonal propagation of banana using axillary branching are usually conducted by culturing shoot tips from rhizome on media containing cytokinins to induce shoot multiplication, subculture each shoot on media containing auxins to promote rooting;and acclimatize the plantlets (Yusnita et al., 2015; Yusnita, 2015). At the base of proliferating shoots sometimes appear scalps, a structure containing clumps of meristematictissue showing an embryogenic characteristic. Under favorable condition scalps could grow into multiple shoots.This research aimed to investigate effects of thidiazuron and benzyladenin that have strong cytokinin activity on shoot multiplications and scalp formation in tissue culture of banana cv Kepok Kuning and Raja Bulu.

MATERIALS AND METHODS

Experiment 1: Response of shoot tips of banana cv Kepok Kuning to concentrations of thidiazuron and benzyladenine

In this experiment, shoot tips of sword suckers as explants were cultured aseptically on a precondition medium(P) for 2 weeks and subcultured onto shoot-inducing media (SI) with several subcultures. After for weeks in culture, the shoot tips were longitudinally sliced four times down to meristem tips to suppress apical dominance. After 10 weeks in culture, shoots and embrygenic nodules (scalps) originated from the base of the explants were observed. The shoots were induced to form roots and the plantlets were acclimatized. The embryogenic nodules were subcultured for proliferationin embryogenic-nodul-proliferating media (NP).

Plant materials were derived from banana plant cv. Kepok Kuning from Lampung Province. Sword suckers with the rhizome of approximately 10-15 cm in diameter were used as source of explants. The pseudostems were separated from the rhizome in such a way that the apical shoots were still intact with the pseudostems. The pesudostems were cut off and the sheaths were peeled off, leaving shoot tip of 15 cm in length, which were then rinsed under running tap water and soaked for 30 minutes in a solution containing 2 g/l fungicide mankozeb and 150 mg/l ascorbic acid, and rinsed again under running tap water. Sheath peeling were done again and the shoots were cut to the size of 5 cm in length and rinsed under running tap water. The sterilization was continued by shaking the shoots in 2.13% NaOCl solution added with 5 drops of Tween-20. Sheath peeling and cutting was done again until the shoots were 1.5 x 1.5 x 1 cm (W x L X H) in size. The shoots were soaked for 15 minutes in ascorbic acid solution (150 mg/l), sterilized for 10 minutes in 0.8% NaOCl solution under vacumed condition in a dessicator and then rinsed three times with sterile distilled water.The procedure was repeated one more and the shoots as explants were ready for culture initiation. Initially the explants were aseptically cultured on P medium and then transfered to SI medium.

The P medium was composed of MS (Murashige and Skoog, 1962) salts, 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glyciene, 30 g/l sucrose,2 mg/l BA, 0.005 mg/l TDZ; 50 mg/l citric acid, and 150 mg/l ascorbic acid. The SI medium was the same as the P medium except that the added plant growth regulatorswere combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (BA) (0 and 2 mg/l).The NP medium was the same as the P medium except that the supplemented plant growth regulator were 0.1 mg/l TDZ.The pH of medium was adjusted to 5.8 by adding HCl 1 N or KOH 1 N. The medium was added with 8 g of agar as solidifying agent,boiled and then dispensed into 250-ml culture bottles, 25 ml/bottle. The

bottles containing the medium were capped with transparent plastic sheets and autoclaved at a pressure of 1.2 kg/cm^2 and a temperature of 121^{o}C for 10 minutes.

Shoot tips as explants were initially cultured on P medium for 2 weeks and then subcultured to SI medium.The SI mediumwassupplemented with combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (0 and 2 mg/l). The treatments were arranged in a completely randomized design with 5 replications, 5 culture bottles per replication, 1 explant per bottle. All cultures were incubated in a culture room at 25 ± 2^{o}C, under continous light of fluorescent lamps of approximately 1000 lux, and After 10 weeks in culture, data on shoots were recorded and embryogenic nodules were separated and cultured on NP medium containing 0.1 mg/l TDZ.Data were subjected to analysis of variance and difference between two data due to treatments was subjected to a least-siginificant difference (LSD) analysis.

Experiment 2: Response of embryogenc nodules cv. Raja Bulu to concentrations of thidiazuron and benzyladenine

Embryogenic nodules were initiated from proliferating axillary shoots of banana cv. Raja Bulu reported by Yusnita *et al* (2015) by culturing the shoots on media MS media containing 0.05 mg/L of TDZ for 8 weeks (Fig.). The embryogenic nodules were treated as previously dercribed using SI medium supplemented with combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (0 and 2 mg/l). Media preparation and culture condition was as previously described. After 8 weeks in culture the proliferating embryogenic nodules were observed and recorded.

RESULTS AND DISCUSSION

Results of Experiment 1 showed that different concentrations of TDZ had significant effects on number of shoots and propagules (shoots and shoot buds), while BA had no significant effects. Both TDZ and BA did not significantly affect number of shoot buds. Interaction of both factors did not significantly influence all of the variables. Increase in TDZ concentrations from 0.9 - 0.1 mg/l resulted in an increase in numberof shoots and propagules (Figure 1). Shoots were defined as those having length 0.5 cm or more, while shoot buds are those having length less than 0.5 cm. Highest number of shoots was attained at treatment of 0.05 mg/l TDZand propagules at 0.1 mg/l TDZ (Figure 1). Based on LSD analysis, number of shoots and number propagules at 0.05 mg/l TDZ and 0.1 mg/l TDZ were not significantly

different, so 0.05 mg/l TDZ was considered the best treatment for maximum shoot proliferation.

Figure 2 showed that treatment 0.1 mg/l TDZ and 0.1 mg/l TDZ + 2 mg/l BA resulted in formation of embryogenic nodules (scalp) in addition to shoots. The nodules from 0.1-mg/l-TDZ treatment were then subcultured onto the same medium for proliferation. Subcultures were conducted every 4 weeks. Subculture started with embryogenic nodule clumps of 0.5 cm, each then proliferated. After 12 weeks, the culture produced 42 clumps containing 433 embryogenic nodules (Figure 3)

Experiment 2 was conducted to confirm whether embryogenic nodules of cultivar Raja Bulucould also proliferate as cv. Kepok Kuning did when they were cultured on media containing TDZ or TDZ + BA. Results of the experiment revealed that all treatment led to proliferation of embryogenic nodules. Range ofTDZ concentrations (0.005 – 0.1 mg/l) resulted in non-significantly different number of nodules, while additions of 2 mg/l BA significantly suppress nodule proliferation (Figure 4 and 5). Therefore, MS medium supplemented with 0.005 mg/l TDZ was optimum for embryogenic nodule proliferation.

The data showed that shoot multiplication response varied with TDZ concentrations. In general the higher the TDZ concentrations the more shoots or the propagules were formed. Even though there was no control treatment i.e. without growth regulator, based on experience and many reports, no shoot multiplication occured in shoot tip culture of banana in media devoid of growth reguators. Therefore, it could be concluded that in this experiment TDZ could induce shoot multiplicationof banana cv. Kepok Kuning, indicating that TDZ showed a cytokinin activity in banana tissue culture. TDZ has beenalso reported to have cytokinin activity in other plants , that was to induce shoot de novo or shoot multiplication in other plants, for example in tissue culture of *Viola odorata* (Mokhtari et al., 2015), *Aloe vera* L. (Lavakumaran and Seran, 2014), *Vitex trifolia* L. (Ahmed and Anis , 2014), *Crocus sativus* L. (Sharifi et al., 2010), *Curcuma vamana* L. (Beyoy et al., 2012), *Saussurea involucrata*Kar. et Kir. (Guo et al., 2012), *Curculigo latifolia* L. (Babaei et al., 2014), *Populus ciliata* Wall.(Aggarwal et al., 2012), and *Salix tetrasperma* Roxb. (Khan and Anis, 2012).

The mode of action of cytokinin activity of TDZ, which was initially produced as a defoliant, has not been conclusively elucitated. However, a group of researchers showed that shoot differentiation response to TDZ had been correlated with an increase in a compound having cytokinin activity such as N^6-(Δ^6-isopentenyl)adenine (iP)(Casanova et al., 2004).

This increase might be due to inhibition of cytokinin oxidase by TDZ, so that more iP was available to tissue. Casanova et al. (2004) did not exclude the posibility that TDZ had its own biological activity based on other reports that receptor proteins for both adenine-type and phenylurea cytokinins had been discovered.

In Experiment 1, TDZ not only induced shoot multiplication but also the formation of scalps, structures containing meristematic nodules. Scalps were considered to be embryogenic bacause they could be turned into embryogenic callus and then turned into somatic embryos (Sadik et al., 2015). In our experiment, the scalps were induced in media containing TDZ, and were then proliferating when cultured on media containing the same regulator (Figure 3).

That TDZ induced the formation of scalp, which was embryogenic in nature, indicates that TDZ was correlated with auxin activitysince auxins has been known to induce somatic embryogenesis. Guo et al (2011) reviewed biochemical and biophysical responses of plant cell to TDZ. They made a list of 58 families of plants that has been reported to respond to TDZ. While most of the families on the list showed shoot formation response, 13 families showed somatic embryogenesis response (Guo et al., 2011), meaning that this was related to endogenous auxin metabolism. Using leaves of *Echinacea purpurea* L. cultured in vitro as a model system, it was demonstrated that TDZ-induced regeneration was correlated with level of auxins (indole-3-acetic acid) in the regenerant tissue. Number of regenerant and auxin level increased by exposure to TDZ. Number of TDZ-induced regenerant was decreased by exposure to auxin transport inhibitor, 2,3,5-triindobenzoic acid (TIBA). Number of TDZ-induced regenerant was also decreased by exposure to auxin action inhibitor, p-chlorophenoxyisobutyric acid (PCIB). Those data suggest that TDZ did not act directly to regulate regeneration, but its action was mediated by endogenous auxin.

The optimal concentration of TDZ for propagation of banana cv. Kepok Kuning was 0.05 mg/l TDZ. Number of propagules should be a variable of consideration because number of shoot buds is important since they could be turned into shoots. In addition, lower concentrations poses less genetic abberation, a requirement for clonal propagation. For embryogenic nodule proliferation, 0.1 mg/l TDZ was found to be optimum for cv. Kepok Kuning and 0.005 mg/l TDZ for cv. Raja Bulu.

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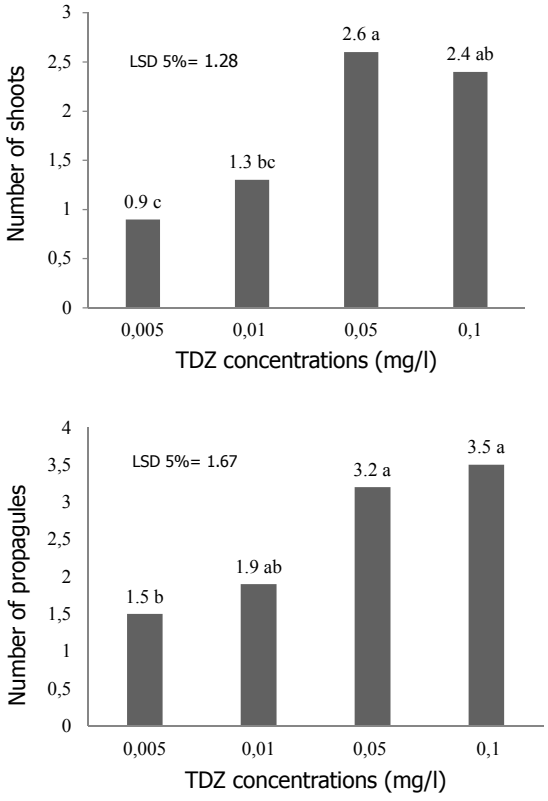

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Figure 1. Effects of thidiazuron on number of shoots (above) and propagules (below) in tissue culture of banana cv. Kepok Kuning after 10 weeks in culture.

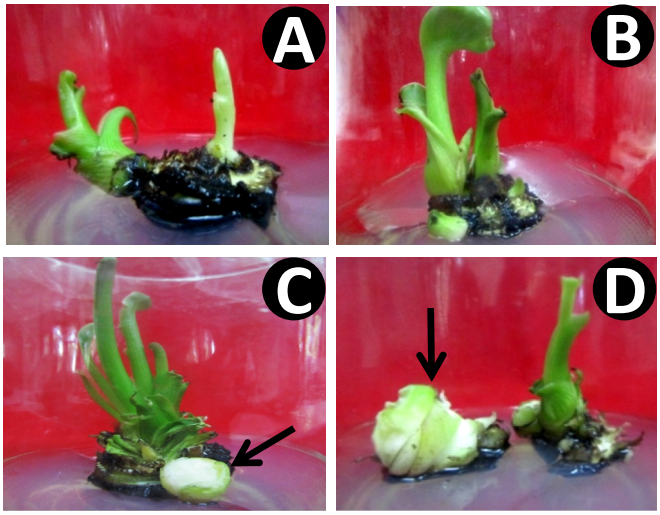

Figure 2. Shoot and scalp (embryogenic nodule) formation (indicated by arrow)in response to thidiazuron (TDZ) and benzyladenine (BA) in tissue culture of banana cv. KepokKuning after 10 weeks in culture. The media was MS containing (A) 0,05 mg/l TDZ. (B)0,05 mg/l TDZ + 2 mg/l BA. (C)0,1 mg/l TDZ. (D) 0,1 mg/l TDZ + 2 mg/l BA.

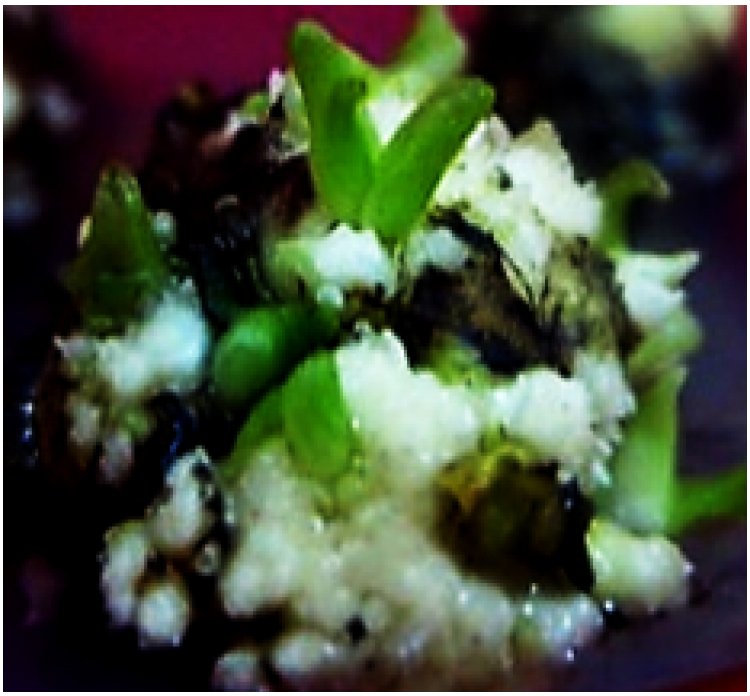

Figure 3. Shoots and proliferating embryogenic nodules in tissue culture of banana cv. Kepok Kuning after 3 subcultures. Subculture was done every 4 weeks. The embryogenicnodules looks white in color. The shoots, that was developed from nodules, appear green in color

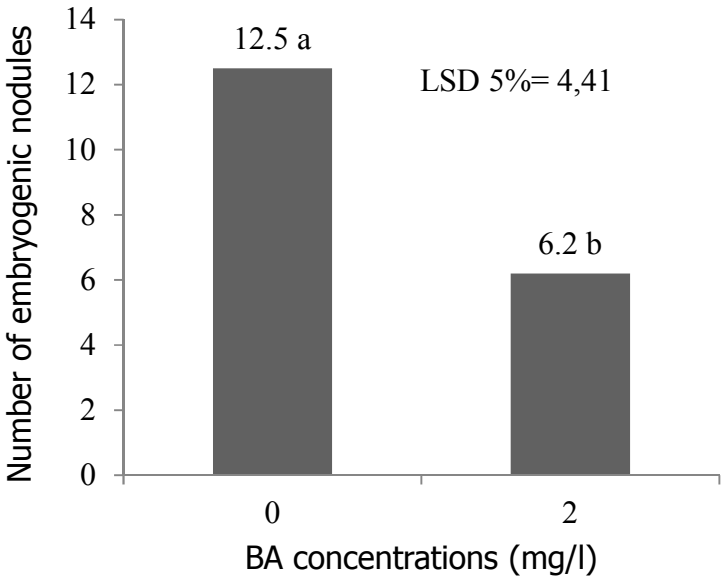

Figure 4. Effects of benzyladenine (BA) on number of embryogenic nodules in tissue culture of banana cv. Raja Bulu.

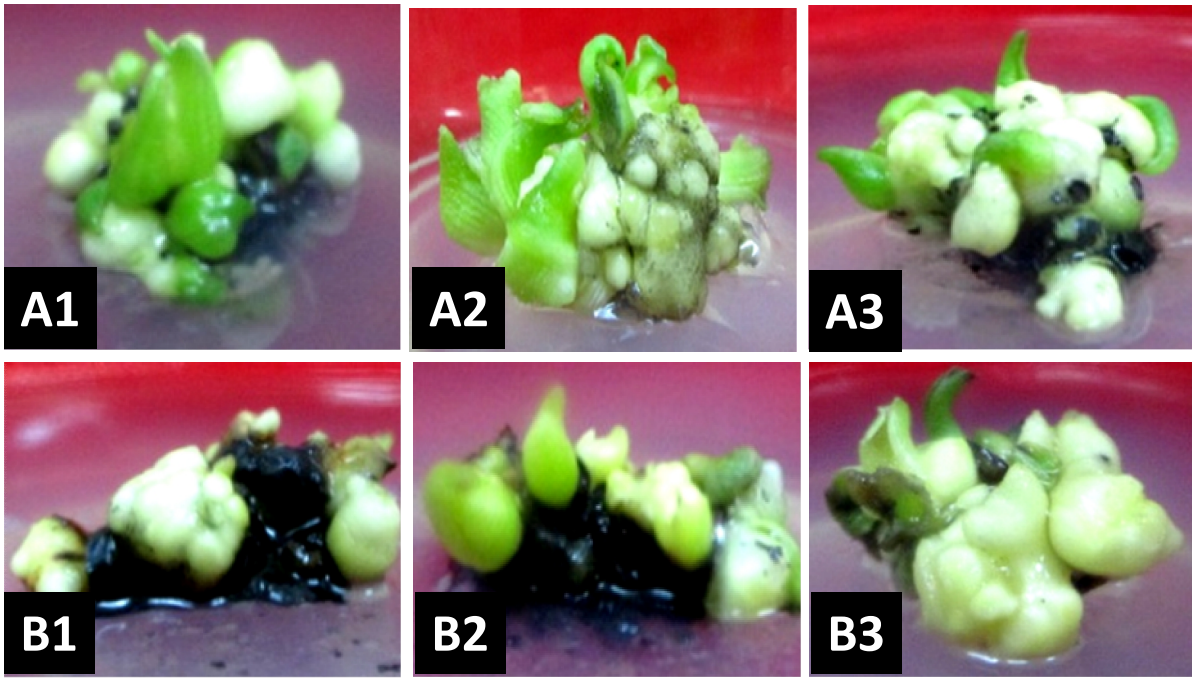

Figure 5. Response of embryogenic nodules to proliferation media containing thidiazuron (TDZ) and benzyladenine (BA) after 8 weeks in culture.Proliferating embryogenic nodules on media containing TDZ (Row A) and TDZ + BA (Row B).(A1) 0,005 mg/l TDZ, (B1) 0,005 mg/l TDZ + 2 mg/l BA, (A2) 0,01 mg/l TDZ, (B2) 0,01 mg/l TDZ + 2 mg/l BA, (A3) 0,05 mg/l TDZ, (B3) 0,05 mg/l TDZ + 2 mg/l BA.