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Planta Tropika focuses related to various themes, topics and aspects including (but not limited) to the following topics Agro-Biotechnology, Plant Breeding, Agriculture Waste Management, Plant Protection, Soil Science, Post Harvest Science and Technology, Horticulture. Planta Tropika published two times a year (February and August) by Universitas Muhammadiyah Yogyakarta in collaboration with Indonesian Association of Agrotechnology / Agroecotechnology (PAGI). The subscriptions for one year : IDR 350.000.

Editorial Address

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Editorial

Journal of Planta Tropika ISSN 0216-499X published by Study Program of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, is journal presenting scientific articles of agricultural science (Journal of Agro Science). With full sense of gratitude to the Almighty Allah, Volume 7 Number 1 for the year of 2019 has been published.

In this edition, Journal of Planta Tropika presents seven research articles in the field of Agro sciences comprising post harvest physiology, crop cultivation system, weeds management, tissue culture, land management, and climate. The scientific articles discuss about:

(1) The Use of Zeolite to Increase Nitrogen Use Efficiency in Corn Vegetative Growth in Coastal Sandy Soils, (2) Effects of Biochar and Chromolaena odorata Liquid Fertilizer Enriched with Sodium Bicarbonate on Soil and Muskmelon (*Cucumis melo* L.), (3) Detection and Identification of Polymorphism in Mutant Strawberry (*Fragaria* spp.) Plants Based on Cleaved Amplified Polymorphic Sequences Molecular Markers, (4) New Promising Rice Genotypes of SP87-1-1-2 and SP73-3-1-7 Adaptive to Lowland and Medium Land, (5) Effects of Chromolaena odorata Compost on Soil and Nutrient Uptake of Lettuce (*Lactuca sativa*), (6) Growth and Yield Responses of Four Soybean (*Glycine max* (l.) Merrill.) Cultivars to Different Methods of NPK Fertilizer Application, (7) Utilization of Diethanolamide Surfactant from Methyl Esters of Palm Oil in Herbicide Formulation with Active Isopropylamine Glyphosate, and (8) Genetic Diversity of Potato Based on Random Amplified Polymorphic DNA and Simple Sequence Repeat Marker.

The editors would like to thank the authors, reviewers, executive editors, leaders and LP3M UMY for their participation and cooperation. Our hope, this journal can be useful for readers or be a reference for other researchers and useful for the advancement of the agriculture.

Editors

GUIDE FOR AUTHORS

TYPE OF PAPERS

PLANTA TROPIKA receives manuscripts in the form of research papers in Bahasa Indonesia or English. The manuscript submitted is a research paper that has never been published in a journal or other publication.

SUBMISSION

The submission of the manuscript is done through our journal website http://journal.umy.ac.id/index.php/pt/index. If you need information regarding the process and procedure for sending the manuscript, you can send it via email at plantatropika@umy.ac.id. Editor's address: Program Studi Agroteknologi, Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta, Jl. Ring Road Selatan, Tamantirto, Kasihan, Bantul, Telp (0274) 387646 psw 224, ISSN: 2528-7079.

ARTICLE STRUCTURE

The submitted manuscripts should consist of 15-20 pages of A4 size paper with 12-point Times New Roman fonts, 1.5 spacing with left-right margin and top-bottom of the paper is 2.5 cm each. All manuscript pages including images, tables and references should be page-numbered. Each table or picture should be numbered and titled.

The systematic of the manuscript writing is as follows:

TITLE : The title should be brief and informative and written bold. Only the first letter of the words is written in uppercase. Maximum length should be 14 words.

AUTHOR NAMES : The author names should be written in lowercase letters (only the first letter of the words is written in uppercase) and should be written from the first author and followed by the others along with the marker of each author's affiliation.

AUTHOR AFFILIATIONS : The author affiliation should be written in lowercase letters (only the first letter of the words is written in uppercase) and it is written according to the order of the number marker of each author's affiliation.

EMAIL : Please list one of authors' email address used for paper's correspondence.

ABSTRAK : Abstrak is written in Bahasa Indonesia using single space in a paragraph with maximum length of 200 words. It should contain background, objective, method, results, and conclusion followed by keywords containing maximum of 5 words.

ABSTRACT : Abstract is written in English using single space in a paragraph with maximum length of 200 words. It should contain background, objective, method, results, and conclusion followed by keywords containing maximum of 5 words.

INTRODUCTION : Introduction contains background, hypothesis or problem outline, and the objective of the research.

MATERIALS AND METHOD : Explaining in detail about materials and method used in the research as well as the data collection and analysis.

RESULT AND DISCUSSION : The results of the research should be clear. State the results collected according to analyzed data. Discussion should include the significance of the results.

CONCLUSION : Authors are expected to give brief conclusion and to answer the objective of the research.

ACKNOWLEDGEMENT : If necessary.

REFERENCES : Single space, according to the authors' guide of Planta Tropika.

EXAMPLES ON HOW TO WRITE REFERENCES

References are written in alphabetical order according to the rules below:

REFERENCE TO A BOOK

Gardner, F.P., R.B. Pearce, and R.L. Mitchell. 1991. Fisiologi Tanaman Budidaya (Translated by Herawati Susilo). UI Press. Jakarta.

REFERENCE TO A JOURNAL PUBLICATION

Parwata, I.G.M.A., D. Indradewa, P.Yudono dan B.Dj. Kertonegoro. 2010. Pengelompokan genotipe jarak pagar berdasarkan ketahanannya terhadap kekeringan pada fase pembibitan di lahan pasir pantai. J. Agron. Indonesia 38:156-162.

REFERENCE TO A THESIS/DISSERTATION

Churiah. 2006. Protein bioaktif dari bagian tanaman dan akar transgenic Cucurbitaceae serta aktivitas antiproliferasi galur sel kanker *in vitro*. Disertasi. Sekolah Pascasarjana. Institut Pertanian Bogor. Bogor.

REFERENCE TO AN ARTICLE IN PROCEEDING

Widaryanto dan Damanhuri. 1990. Pengaruh cara pengendalian gulma dan pemberian mulsa jerami terhadap pertumbuhan dan produksi bawang putih (Allium sativum L.). Prosiding Konferensi Nasional X HIGI hal. 376-384.

FIGURE FORMATTING

Title should be given **below each figure**. Additional information (notes) should be written in lowercase letters except the first letter in each sentence. All figures need to be numbered respectively. Figures should be placed close to explanation/ discussion about the figure.

Examples :

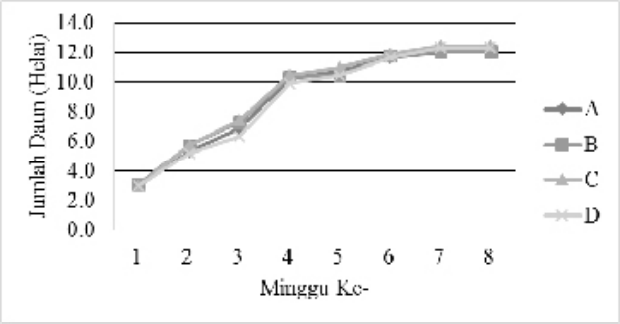

Figure 1. Number of leaves of corn plant

Notes:
A = 250 kg KCl/ha + 0 kg KJP/ha
B = 125 kg KCl/ha + 273,89 kg KJP/ha
C = 62,5 kg KCl/ha + 410,84 kg KJP/ha
D = 0 kg KCl/ha + 547,79 kg KJP/ha

Fig. 1., Fig. 2., and so on. The title of the figure is written with lowercase letters (use uppercase letter at the beginning of the title only) and without full stop (.). Additional information (notes) is placed below the figure.

TABLE FORMATTING

The title of the table should be written **above the table** started from the left (left alignment). Additional information related to the table (notes) is placed below the table. The information is written in uppercase letters at the beginning only as well as the titles inside the table. Table is placed close to the discussion of the table.

Examples :

Table 1. Fruit compost analysis

Variable	Jatropha before composted	Jatropha after composted	SNI (National standard) for compost	Category
Water content	22,49 %	45,79 %	≤ 50 %	Qualified
pH	7,05	8,02	4-8	Qualified
C-Organic content	10,01	5,11	9,8-32 %	Not qualified
Organic matter	17,42 %	8,81 %	27-58	Not qualified
N-Total	0,97 %	2,69 %	< 6 %	Qualified
C/N Ratio	10,44	1.90	≤ 20	Qualified
Potassium	-	9,06 %	< 6 %**	Qualified

Notes: **) Certain materials originated from natural organic matters are allowed to contain P_2O_5 dan K_2O level > 6% (proved with the results of laboratory analysis).

The Application of Zeolite to Increase Nitrogen Use Efficiency in Corn Vegetative Growth in Coastal Sandy Soils

DOI: 10.18196/pt.2020.107.1-6

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ABSTRACT

Coastal sandy soil is usually dominated by sand fractions, having no micro pore complex that can bind water and store fertilizer nutrients. The application of zeolite rocks into the root zone of plants growing in sandy soil is expected to reduce nitrogen nutrient leaching.The research was carried out by using the greenhouse experiment method, arranged in a factorial completely randomized design. The first factor was activated zeolite rock doses, consisting of 7 levels, namely 0% (Z0), 1% (Z1), 2% (Z2), 3% (Z3), 4% (Z4), 5% (Z5), and 6% (Z6) per 10 kg of coastal sandy soils as planting media. Meanwhile, the second factor was nitrogen fertilizer doses, consisting of 3 levels, namely 75 kg (N1), 100 kg (N2), and 125 kg (N3) per hectare. The results showed that the application of zeolite rock to the planting media could increase the growth of corn plants fertilized with nitrogen. The best vegetative growth was obtained when 6% zeolite per 10 kg of planting media was applied. The application of 6% zeolite together with 125 kg per hectare N fertilizer resulted in the heaviest fresh plant biomass. The use of zeolite can increase the N-fertilizer uptake efficiency in the vegetative growth of corn plants grown in coastal sand soils.

Keywords: Low water retention; Sand fraction dominance; Nitrogen leaching, Aluminosilicate minerals

ABSTRAK

Tanah pasir pantai biasanya didominasi oleh fraksi pasir, sehingga tidak memiliki kompleks pori mikro yang dapat mengikat air dan menyimpan hara pupuk. Aplikasi batuan zeolite ke dalam zona akar tanaman yang tumbuh di tanah pasir diharapkan dapat mengurangi pelindian hara nitrogen. Penelitian ini dilaksanakan menggunakan metode percobaan rumah kaca yang disusun dalam rancangan acak lengkap faktorial. Faktor pertama adalah batuan zeolite teraktifasi terdiri 7 level yaitu 0%(Z0), 1%(Z1), 2%(Z2), 3%(Z3), 4%(Z4), 5%(Z5) dan 6%(Z6) per berat 10 kg media tanah pasir pantai. Sedangkan faktor kedua adalah pupuk nitrogen yang terdiri 3 level yaitu 75 kg per hektar (N1), 100 kg per hektar (N2) dan 125 kg per hektar (N3). Hasil penelitian menunjukkan bahwa aplikasi batuan zeolite ke dalam media tanam tanah pasir dapat meningkatkan pertumbuhan tanaman jagung yang dipupuk nitrogen. Dosis 6% zeolite per 10 kg media tanam tanah pasir pantai menghasilkan pertumbuhan vegetatif paling baik. Kombinasi perlakuan 6% zeolite dan 125 kg N per hektar menghasilkan biomassa segar tanaman paling berat. Pemanfaatan zeolite dapat meningkatkan efisiensi serapan hara N-pupuk pada pertumbuhan vegetatif tanaman jagung di tanah pasir pantai.

Kata Kunci: Retensi air rendah, dominasi fraksi pasir, pelindian nitrogen, mineral aluminosilikat

INTRODUCTION

Coastal sandy soil is one form of marginal land that has low productivity. This type of soil contains low available nutrients and has a low ability to store water due to the dominance of sand fraction. Low clay mineral and organic matter content cause the soil not to form aggregates, with a weak structure or even without structure (Bruand et al., 2014), so it does not have soil colloids as nutrient binding complexes.

The coastal sandy soil area of Bugel beach Kulon Progo is part of more than 10,000 hectares of sandy beach stretching along the southern coast of Bantul and Kulon Progo. Bugel sandy beach has an area of around 3,000 hectares, and some parts of the area have been used by residents to cultivate various types of agricultural commodities such as watermelons, chilies, corn, and vegetables. One of the main problems of these soils is the low moisture content due to their high infiltration capacity and soil permeability. This condition causes the water not to be able to remain in the pores of the soil, moving out of the rooting zone. This process results in low moisture content in the root zone, thus cannot support plant growth. Hoa et al. (2010) state that sandy soils have many factors that limit agricultural production, including nutrient short-

ages and low water storage capacity.

Soil dominated by sand fraction with low clay mineral and organic matter content has high porosity and easily escapes water out of the root zone. Therefore, crop cultivation in coastal sand has low nitrogen fertilization efficiency, which is due to the unavailability of colloidal complexes of soils capable of binding nitrogen ions of the fertilizer. Dry coastal sandy soil conditions make the root zone space aerobic and stimulate the transformation of ammonium ions into nitrate ions in the soil solution system, making it easily leached (Budiyanto, 2016). This nitrogen leaching process is an obstacle to successful nitrogen fertilization in coastal sandy soils.

The productivity of sandy soil can be improved by adding materials that can increase water storage capacity. Some treatments include the addition of clay minerals, application of organic matter, mulching, and the use of other materials that can store water. The treatments aim to improve the status of water in the root zone and reduce the rate of gravity water so that it can support the nutrient uptake process. The improvement can also be achieved by adding a soil amendment that can bind water and nutrients and gradually free up nutrients for plants to absorb. Utilization of hydrogel as a soil amendment material in sandy soil by Akhter et al. (2004) proved that the material could delay plant wilting and increase soil moisture content. In addition, Tahir and Marschner (2016) used clay minerals as soil ameliorant that was proven to increase crop production in sandy soils.

One of the soil amendment materials is zeolite rock, a form of a hydrated alumina-silicate compound that has multi structural properties. This mineral has water-absorbing properties and acts as ion exchange. Zeolite can be used in agriculture as a soil softener, nutrient carrier, regulator of nutrient release in soil, and agent to increase the diversity of microorganisms in the soil (Sugiarti and Amiruddin, 2008; Sangeetha and Baskar, 2016; Mahesh et al., 2018). Furthermore, the Director-General of Food Crops and Horticulture (1998) states that zeolite is able to increase the solubility of dissolved oxygen in paddy fields, maintain soil pH stability, and fix heavy metals such as Pb and Cd. The addition of zeolite causes the soil to retain fertilizer cations (NH_4^+-urea and K^+-KCl), thereby making them not easily leached by the movement of gravity water as well as increasing soil CEC and crop yields. In addition, the application of zeolite can also increase nutrient uptake of nitrogen and phosphorus in rice cultivation. (Zheng et al., 2019; Wu et al., 2019).

MATERIALS AND METHODS

In this research, coastal sandy soils were used as planting media, which were taken from Bugel beach Kulon Progo at a depth of 40 cm. Meanwhile, zeolite rocks were mined from Playen Gunung Kidul. The research was conducted from April to August 2019 in the experimental field of the Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta.

The research was carried out using the greenhouse experiment method, arranged in a completely randomized factorial design (Harsojuwono et al., 2011). The first factor was activated zeolite rock doses, consisting of 7 levels, namely 0% (Z0), 1% (Z1), 2% (Z2), 3% (Z3), 4% (Z4), 5% (Z5), and 6% (Z6) per 10 kg of sandy soils as planting media. The second factor was nitrogen fertilizer doses, consisting of 3 levels, namely 75 kg (N1), 100 kg (N2), and 125 kg (N3) per hectare. There were four replications within each treatment combination, resulting in a total of 84 experimental units.

Observation on the growth variables of the corn plants was performed eight weeks after planting or during the vegetative growth phase marked by the

emergence of primordial flowers. Growth variables observed were plant height, plant stem diameter, and fresh weight and dry weight of plant biomass. The data obtained were analyzed using analysis of variance, followed by Duncan's multiple range test at a level of 5%.

RESULTS AND DISCUSSION

The effect of zeolite application on water and nitrogen availability can be observed from the vegetative growth of corn plants at eight weeks after planting. The analysis of variance indicated that there was no significant interaction effect of the zeolite and nitrogen fertilizer doses on the plant height (Table 1).

Table 1. Plant Height (cm) at Eight Weeks After Planting

Zeolite doses (%)	Nitrogen fertilizer doses			Mean
	N1 (75 kg/h)	N2 (100 kg/h)	N3 (125 kg/h)	
Z0 (0)	66.45	78.75	86.50	77.23 e
Z1 (1)	95.70	112.25	100.75	102.90 d
Z2 (2)	110.20	124.70	125.75	120.22 c
Z3 (3)	124.50	126.75	135.00	128.75 bc
Z4 (4)	115.25	128.25	148.70	130.73 bc
Z5 (5)	115.10	128.75	158.50	134.12 b
Z6 (6)	150.20	168.20	176.75	165.05 a
Mean	111.06 q	123.93 p	133.14 p	(-)

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%.

Table 2. Stem Diameter (cm) at Eight Weeks After Planting

Zeolite doses (%)	Nitrogen fertilizer doses			Mean
	N1 (75 kg/h)	N2 (100 kg/h)	N3 (125 kg/h)	
Z0 (0)	1.10	1.20	1.18	1.16 c
Z1 (1)	1.20	1.25	1.15	1.20 c
Z2 (2)	1.25	1.25	1.35	1.28 bc
Z3 (3)	1.28	1.30	1.35	1.31b
Z4 (4)	1.35	1.35	1.38	1.36 b
Z5 (5)	1.40	1.40	1.45	1.42 ab
Z6 (6)	1.45	1.45	1.75	1.55 a
Mean	1.29 p	1.31 p	1.37 p	(-)

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%.

Table 1 shows that increasing doses of zeolite can improve plant height. The application of 6% zeolite per weight of sandy soils resulted in the highest corn plants with a height of 165.05 cm. Likewise, increasing doses of nitrogen fertilizer up to 100 kg per hectare can also increase plant height.

There was no interaction between zeolite and nitrogen fertilizer doses in affecting the growth of plant corn. Increasing the dose of zeolite is thought to increase water supply in the root zone and support the process of nitrogen nutrient uptake. The results of plant height measurements at an average dose of nitrogen fertilizer showed that the highest dose of zeolite (6% per weight of 10 kg of sandy soils) produced the highest plants. This result is following the result by Sangeetha and Baskar (2016), reporting that the application of zeolite as a soil amendment material could increase the availability of water for plants in sandy soil. Besides, zeolite is beneficial in reducing the leaching rate of N element because the negative charge side of the zeolite surface can bind NH_4^+ ions (Omar et al., 2015), and reduce the transformation process to NO_3^- mobile ions in the soil solution system.

The same result was also observed on the stem diameter of the corn plants. The doses of zeolite and nitrogen fertilizer did not give significant interaction effect on the stem diameter of the corn plants (Table 2).

There was a significant effect of zeolite doses on the stem diameter of the corn plants. The application of 6% zeolite per weight of the sandy soils as planting media produced the largest stem diameter. Meanwhile, the doses of nitrogen fertilizer did not significantly affect the stem diameter of the corn plants.

Tables 1 and 2 show that at various doses of nitrogen fertilizer, an increase in the dose of zeolite can increase the availability of water for the nitrogen uptake process that occurs in the root zone. Zeolite is a hydrated aluminum mineral that

has a three-dimensional structure formed by the tetrahedral $(SiO_4)^{-4}$ and $(AlO_4)^{-5}$ structures. These three-dimensional structures are interconnected so that they have canals and tunnels, and are generally negatively charged so that they can bind cations (Sugiarti and Amiruddin, 2008; Lestari, 2010). Therefore, zeolite application can improve the ability of sandy soils to store water and improve nutrient balance in sandy soils (Al-Busaidi et al., 2008).

The observation on the fresh weight of corn biomass showed that there was a significant interaction effect of zeolite and nitrogen fertilizer doses on vegetative growth. The effect of the combined treatment of zeolite and nitrogen fertilizer doses is presented in Table 3.

Table 3. Fresh Weight of Corn Plant Biomass (gram) at Eight Weeks After Planting

Zeolite doses (%)	Nitrogen fertilizer doses			Mean
	N1 (75 kg/h)	N2 (100 kg/h)	N3 (125 kg/h)	
Z0 (0)	58.5 k	86.4 jk	110.6 ijk	85.2
Z1 (1)	121 hijk	156.6 efghi	202.1 cdefg	159.9
Z2 (2)	137.7 ghij	165.8 defghi	161.9 efghi	155.1
Z3 (3)	165.3 defghi	174.3 cdefghi	146.5 fghij	162.0
Z4 (4)	156.8 efghi	170.4 cdefghi	238.8 c	188.7
Z5 (5)	211.2 cdef	226.1 cde	234.4 cd	223.9
Z6 (6)	183. 4 cdefgh	308.6 b	435.7 a	309.2
Mean	147.7	184.0	218.6	(+)

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%.

The application of nitrogen fertilizer at all doses (75, 100, and 125 kg per hectare) without zeolite treatment produced the lowest weight of fresh biomass compared to other treatment combinations. Meanwhile, the application of 100 and 125 kg of nitrogen fertilizer per hectare, when combined with 6% zeolite per weight of the growing media, produced higher biomass fresh weight than the application of 75 kg of nitrogen per hectare.

The fresh weight of plant biomass is the weight of the entire plant tissues that have been developing for a certain time, along with the water contained. Plants that have better growth have cells containing more water. Table 3 shows that the treatment combination of zeolite and nitrogen fertilizer can improve the growth of plant cells and tissues. Zeolite can increase water supply and nitrogen nutrient uptake for the growth of plant cells and tissues. The addition of zeolite can suppress the rate of nitrification due to NH_4+ ions and nitrogen leaching because it is bound by its specific surface (Ippolito et al., 2011), and this condition supports the process of nitrogen nutrient uptake of plants. The ability of zeolite to fix NH_4+ ions and delay nitrification was also conveyed by Sudirja et al. (2016), reporting that mixing Urea and Zeolite (95:5) could increase the total nitrogen content of the soil.

Analysis of variance on the dry weight of plant biomass showed that there was no significant interaction effect of zeolite and nitrogen fertilizer doses on plant growth. However, each treatment significantly affected the dry weight of plant biomass (Table 4).

Increasing the dose of zeolite can significantly increase the dry weight of plant biomass. These data show that the availability of water in the root zone will guarantee the process of nitrogen nutrient uptake for the growth and development of corn plant cells and tissues. It is suspected that increasing the zeolite dose can create water sorption complexes in the root zone so that gravity water can be reduced, and the leaching of nitrogen compounds will also be reduced. The addition of zeolite turned out to be able to improve the insufficiency of sandy soil as a planting medium. The use of zeolite will increase water storage capacity and cation exchange capacity so that N-ammonium nutrients can be stored. Experiments carried out by Aina et al. (2017) showed that the use of zeolite in corn plantations could increase the biomass of corn plants cultivated using NPK. Another experi-

Table 4. Dry weight of corn plant biomass (gram) at eight weeks after planting

Zeolite doses (%)	Nitrogen fertilizer doses			Mean
	N1 (75 kg/h)	N2 (100 kg/h)	N3 (125 kg/h)	
Z0 (0)	9.65	12.80	14.78	12.41 d
Z1 (1)	17.90	21.85	22.24	20.66 c
Z2 (2)	23.54	24.78	24.90	24.41 bc
Z3 (3)	24.45	21.40	27.95	23.60 bc
Z4 (4)	25.35	29.45	23.15	25.98 b
Z5 (5)	26.46	26.18	33.18	28.61 b
Z6 (6)	32.90	37.45	50.75	40.37 a
Mean	22.89 q	24.84 q	28.14 p	(-)

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%.

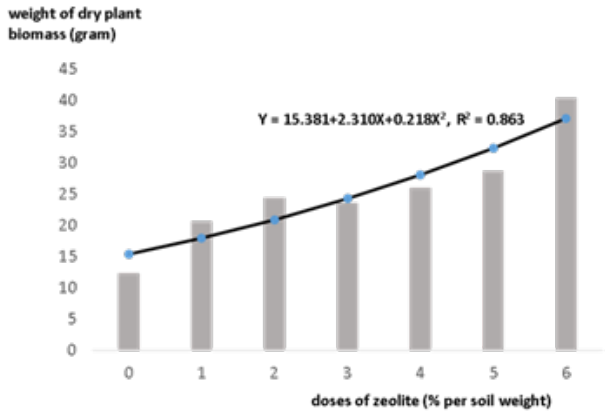

Figure 1. Effect of the Increasing Dose of Zeolite on the Dry Weight of Plant Biomass at Average Doses of Nitrogen Fertilizer

ment proved that the combination of 750 kg zeolite per hectare and 50 kg urea per hectare showed the best results on leaf area, plant height, and total dry weight of plants compared to 300 kg urea per hectare without zeolite (Widyanto et al., 2013).

The dry weight of plant biomass is the net weight of photosynthesis, largely determined by how much nutrients the plant can absorb. The fertilizer dose of 125 kg per hectare produced the highest dry weight of plant biomass. On the other hand, 125 kg of nitrogen fertilizer per hectare is the optimum dose for the growth of corn plants. The comparison of the dry weight of plant biomass produced by the three treatments shows that the addition of nitrogen fertilizer up to a certain dose can guarantee a better growth of plant cells and tissue.

Table 4 also shows that increasing the dose of zeolite at the average doses of nitrogen fertilizer can significantly increase the dry weight of plant biomass. The relationship between increasing zeolite dose at the average doses of nitrogen fertilizer and the increase of plant biomass dry weight is shown in Figure 1.

Figure 1 shows that at the average doses of nitrogen fertilizer, zeolite application can increase the dry weight of plant biomass. The presence of zeolite in the root zone proves to be able to increase water availability and nitrogen nutrient fixation, as well as to increase cell and plant tissue growth. The estimated curve on the effect of zeolite doses on plant biomass dry weight was formulated into a quadratic non-linear curve relationship pattern of $Y = 15.381 + 2.310X + 0.218X^2$ with a determinant coefficient (R^2) of 0.863. The curve shows that increasing the zeolite dose to above 6% per weight of the sandy soil could still increase the dry weight of plant biomass. The coefficient of determination of 0.863 indicates that an 86.3% increase in dry weight of plant biomass is determined by an increase in zeolite dose. The use of zeolite in the cultivation of annual crops such as mustard greens and sorghum can significantly increase the efficiency of nitrogen uptake (Bhaskoro et al., 2015; Suminarti, 2019).

CONCLUSION

The application of zeolite minerals to coastal sandy soils as planting media could increase the growth of nitrogen-fertilized corn plants. A dose of 6% zeolite per weight of 10 kg of coastal sandy soils as planting media produced the best vegetative growth. The combination of 6% zeolite and 125 kg N per hectare resulted in the highest fresh weight of plant biomass. Thus, the use of zeolite can increase the efficiency of nutrient uptake of N-fertilizer in the vegetative growth of corn plants in coastal sandy soils.

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Effects of Biochar and *Chromolaena odorata* Liquid Fertilizer Enriched with Sodium Bicarbonate on Soil and Muskmelon (*Cucumis melo* L.)

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ABSTRACT

Biochar is an organic material instantly made by burning wood into charcoal by pyrolysis, which can meet the high demand for an organic material that cannot be available in a short time. The study aimed to determine the effect of biochar and Chromolaena odorata liquid fertilizer (CLF) enriched with Sodium bicarbonate on soil pH and Muskmelon (Cucumis melo L.) productivity. The research was conducted from December to February 2019 at the field station of Tamansiswa University, Padang. The study was carried out in a factorial experiment with 2 treatment factors arranged in a Completely Randomized Design, consisting of three replications within treatments. The data obtained were analyzed using the F test at 5%, followed by the Least Significant Difference (LSD) test at 5%. The results showed that there was an interaction effect of Biochar and CLF application on reducing the soil pH. Among all treatments, the application of 2 t.Ha^{-1} biochar combined with 50 ml.L^{-1} CLF + 0 g.L^{-1} sodium bicarbonate resulted in the highest growth rate and yield of muskmelon in Ultisol soil.

Keywords: Biochar; C. odorata; Muskmelon; Sodium bicarbonate; Ultisol

ABSTRAK

Biochar merupakan bahan organik yang dibuat secara instan dengan membakar kayu menjadi arang secara pirolisis. Hal ini untuk menjawab tingginya kebutuhan bahan organik yang tidak bisa tersedia dalam waktu singkat. Percobaan ini bertujuan untuk mengetahui pengaruh Biochar dan pupuk cair *C. odorata* (PCC) yang diperkaya oleh Sodium bicarbonat terhadap sifat kimia tanah (pH), pertumbuhan dan hasil melon (*Cucumis melo* L.). Penelitian telah dilakukan pada bulan Desember- Februari 2019, di lahan percobaan Universitas Tamansiswa Padang. Percobaan dilaksanakan dalam bentuk Faktorial dengan 2 faktor perlakuan disusun dalam Rancangan Acak Lengkap, dan 3 ulangan. Data yang diperoleh dilakukan analisis menggunakan uji F taraf nyata 5%, dan dilanjutkan dengan uji Beda Nyata Terkecil (BNT) taraf nyata 5%. Dari hasil percobaan maka disimpulkan bahwa ada interaksi pemberian biochar dan PCC dalam menurunkan pH rizosfer tanaman melon secara nyata. Pemberian 50 ml.L^{-1} PCC + 0 g.L^{-1} Sodium Bicarbonate diiringi dengan 2 t.Ha^{-1} biochar memberikan pertumbuhan dan hasil melon tertinggi mencapai 1,30 kg per buah per tanaman pada Ultisol.

Kata Kunci: Biochar; *C. odorata*; Melon; Sodium bicarbonat; Ultisol

INTRODUCTION

Fertilization is important in muskmelon cultivation. High soil organic matter content will result in high melon production as well. Ultisol is a mineral soil with organic matter content, alkaline saturation, and low pH (Soil Management, Fertilizer Use and Crop Nutrition, 2009). Jamilah & Herman (2018) reported that the pH of Ultisol Lubuk Minturun, which was around 4.82, was classified as acidic. However, the procurement of soil organic matter requires a long time (Jamilah, 2010); (Ariyanto, Sickness, Project, & Bisa, 2012); (Anonim, 2015); (Herman, Resigia, & Syahrial, 2018). This problem can be overcome by producing biochar in the form of wood charcoal made by burning wood in a lack of oxygen (pyrolysis). Biochar is very important for soils, especially soils that are low in organic matter content (Gani, 2009). The use of biochar has been reported in a lot of research on various types of plants, both annual and perennial crops, resulting in the soil fertility improvement as well as the increase in plant growth rate and yield (Gani, 2009); (Mawardiana, Sufardi, & Husen, 2013); (Sudjana, 2014). It is even known that the use of biochar can reduce the effects of chemical residues both from artificial fertilizers and excessive use of pesticides, thereby providing health to the environment (Herman et al., 2018).

The use of liquid organic fertilizer is so im-

portant that the use of chemical fertilizers can be reduced slowly. The use of biochar through the soil and organic liquid fertilizer through plant leaves is considered ideal in a fertilization method. Organic liquid fertilizer derived from C. *odorata* raw material has been proven to be able to reduce the use of artificial fertilizers by 25-50% in various food crops and vegetables (Jamilah, 2010); (Jamilah, 2015); (Jamilah, 2016); (Jamilah, 2018). The effectiveness of C. *odorata* liquid fertilizer (CLF) is also possible to be increased by enriching it with sodium bicarbonate. Sodium bicarbonate is already popular abroad to be applied in various crops and places with specific purposes. The provision of small amounts of sodium bicarbonate can even increase the amount of chlorophyll and photosynthetic activity. The application of sodium bicarbonate was also able to increase the sweet taste of tomatoes (Bie, Ito, & Shinohara 2004; Campbell & Nishio 2000). The effect of sodium bicarbonate is unknown if it is used to enrich the nutrient of CLF on increasing the growth and yield of melon plants. The purpose of this study was to determine the effect of biochar and CLF enriched with sodium bicarbonate on soil chemical properties (pH) as well as on the growth and yield of Muskmelons (*Cucumis melo* L.)

MATERIALS AND METHODS

The pot experiments were carried out in the field of Faculty of Agriculture, Tamansiswa University, located in Ampang Padang, about 5 km from campus at 20 m above sea level. The materials and tools used were Ceramic trademark Muskmelon seeds, skyrocket. Fertilizers used were biochar from wood charcoal made by pyrolysis combustion minus oxygen, C. *odorata* liquid fertilizer (CLF) and sodium bicarbonate. Ultisol was used as planting media, which was taken from Lubuk Minturun.

The experiment was carried out in a completely randomized factorial design with 2 factors. The first factor was was the administration of biochar at a dose of 0, 2 t ha^{-1}, and 4 t ha^{-1}. The second factor was the administration of CLF enriched with sodium bicarbonate at a concentration of 0 ml L^{-1}, 50 ml L^{-1} CLF + 0 mg L^{-1} sodium bicarbonate, and 50 ml L^{-1} CLF + 2 g L^{-1} sodium bicarbonate, replicated 3 times to obtain 9 treatment combinations and 27 experimental units. Each treatment was assigned 3 sample observations, resulting in 81 pots.

The fine Ultisol was sifted through a diameter of 2 mm and inserted into a pot with an average weight of 10 kg. PONSKA basic fertilizer (15-15-15) was given at 400 kg ha^{-1}. Fertilizer and biochar measurements were based on a plant population of 40000 per hectare. Biochar was produced by burning wood into charcoal that was then tightly closed before forming ash. After chilling, biochar was mashed and weighed according to the treatments. Biochar was incubated for a week by stirring it evenly on the planting medium and keeping it in a tightly closed black plastic. Liquid fertilizer was made from a mixture of C. *odorata* plants added with coco fiber, manure, cow urine, coconut water, local microorganisms (LOM), and banana stems. The ingredients were decomposed by keeping it in a tightly closed place for a month and then put into a fermentation container with a composition of 90% that was then added with LOM and cow urine until 100%. The next step was adding water with the same ratio as the ingredients to be fermented for 4 months, then filtered and applied as treatments.

Melon seeds were sown on the seedbed and then transferred to each pot after 2 weeks. Liquid fertilizer was applied to the plants every week until the 6th week. Fertilizers and biochar as treatments were given when transplanting from seedling media to pots. The observed variables included soil pH (H2O) and plant length at 21 and 64 days after planting. The measurement of plant length was carried out starting from the base stems to the growing point. The number of branches was determined by

counting all the branches of plants on the primary stem. The weight of fresh crop stover was observed by weighing all fresh stover without melons (fruits). The weight and circumference of the fruit were also determined. The soil pH was determined by using the pH of the electrode of the soil samples taken from the rhizosphere of Melon roots that had been harvested and then air-dried. As much as 10 g of soils were dissolved in 25 ml of water (pH = 6.83) with a ratio (1: 2.5), then shaken +15 minutes, then precipitated 3 minutes and measured for its pH.

Observational data were analyzed statistically using F-tests at 5%. If the treatments showed a significant effect, further tests were carried out using an LSD test at 5%.

RESULTS AND DISCUSSION

There was an interaction effect of biochar and CLF on the chemical reaction of the soil (pH) of the rhizosphere of the melon plant. Data were analyzed chemically and subsequently analyzed statistically using F-tests at 5%. Further test was performed using the LSD test (Table 1).

Combined with biochar application at 0 and 2 t ha^{-1}, the administration of CLF reduced the pH of Ultisol soils from 4.33 to 3.99 (7.8%) and from 4.04 to 3.77 (6.68%), respectively. Meanwhile, the increasing dose of biochar without CLF administration significantly decreased the soil pH in the rhizosphere of melon from 4.33 to 3.87 is (10.62%). This result was also proven by Tambunan, Siswanto, & Handayanto (2014), reporting that the administration of biochar had the effect on reducing soil pH in corn from 6.93 to 6.23 (10%) at 49 days after planting. Combined with 50 ml of L^{-1} CLF + 0 gL^{-1} sodium bicarbonate, the increasing dose of biochar did not significantly reduce soil pH, while when combined with 50 ml L^{-1} CLF + 2 g L^{-1} sodium bicarbonate, it significantly reduced soil pH from 3.99 to 3.66 (8.27%). The administration of CLF also showed an effect on reducing the soil pH in melon rhizosphere. The pH of CLF was adjusted to 8.6 before it was sprayed to the plants. This result showed that there was a metabolic effect contributing more organic acids compared to the plants that were not sprayed with CLF. Reports by (Gani, 2009); (Tambunan et al., 2014); (Nurida, 2014); (Zaylany, 2017) and (Hasibuan, 2017) proved that the biochar of various agricultural waste materials could increase soil pH. Bargmann, Rillig, Buss, Kruse, & Kuecke (2013) proved that high-dose biochar, in general, reduced the germination rate of Barley plants, while the low dose of biochar increased their development. Lehmann et al. (2011) proved that Biochar did not have a negative effect on plant roots and soil biota, nor did it have a positive impact on the chemical properties of Ultisols.

Table 1. Effect of Biochar and CLF enriched with sodium bicarbonate on the pH of the rhizosphere of muskmelon at 64 DAP (days after planting)

Treatments	Provision of CLF + Sodium Bicarbonate			Average
Biochar	0 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 2 g L^{-1}	
		pH (H_2O)		
0 t ha^{-1}	4.33 aA	3.79 aB	3.99 aB	4.03 a
2 t ha^{-1}	4.04 abA	3.72 aB	3.77 abAB	3.84 b
4 t ha^{-1}	3.87 bA	3.90 aA	3.66 bA	3.81 b
Average	4.08 A	3.80 B	3.80 B	
CV (%)	4.67			

Remarks: Means followed by the same uppercase letters in the same row and means followed by the same lowercase letters in the same column are not significantly different according to the LSD test at 5%.

The application of Biochar and CLF to several agronomic parameters of melon plants is presented in Table 2. There was an interaction effect of the biochar and CLF on the plant length at flowering stage and number of branches.

In general, the administration of CLF significantly affected the plant length at harvest and the fresh stover weight. The longer the plant, the heavier the fresh stover, in which the highest value was observed in the treatment of 50 ml L^{-1} CLF + 0 g sodium bicarbonate. The application of biochar showed no significant effect on the plant length at harvest.

The application of 2 t ha^{-1} biochar produced plant length at flowering that was not significantly different from the length of plants are given 4 t ha^{-1} biochar. This result was due to soil pH (Table 1) that was already so low that nutrients were not optimally available for melon plants. (Jennifer & Morgan, 2013); (Oosterhuis, 2009) explains that pH greatly influences the availability of nutrients in the soil. In the pH range of 3-4, in general, some nutrients such as Ca, Mg, K, and Nitrogen will be difficult to be absorbed by plants., but microelements such as Fe and Mn will be highly available. Plants that were given CLF + 0 g Sodium bicarbon-

Table 2. Effects of biochar and CLF enriched with sodium bicarbonate on muskmelon growth

Treatments	Provision of CLF + Sodium Bicarbonate			Average
Biochar	0 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 2 g L^{-1}	
Plant length at 21 days after the first flower (cm)				
0 t ha^{-1}	26.77 aA	30.85 aA	20.27 bB	25.96
2 t ha^{-1}	27.52 abA	28.17 abA	28.60 aA	28.09
4 t ha^{-1}	22.75 bA	25.84 bA	26.50 aA	25.03
Average	25.68	28.29	25.12	
CV (%)	13.18			
Plant length at 64 days after the first flower (cm)				
0 t ha^{-1}	98.58	147.43	122.93	122.98
2 t ha^{-1}	110.26	115.23	114.38	113.29
4 t ha^{-1}	97.37	113.92	114.61	108.63
Average	102.07 B	125.53 A	117.31 AB	
CV (%)	13.62			
Number of branches (strands)				
0 t ha^{-1}	6.00 aB	7.33 aA	6.50 bAB	6.61
2 t ha^{-1}	6.00 aB	7.83 aA	7.00 abAB	6.94
4 t ha^{-1}	6.83 aB	6.00 bB	8.00 aA	6.94
Average	6.28	7.06	7.17	
CV (%)	8.79			
Fresh stover weight of melon plant (kg)				
0 t ha^{-1}	0.21	0.27	0.28	0.25 b
2 t ha^{-1}	0.22	0.35	0.36	0.31 a
4 t ha^{-1}	0.21	0.40	0.36	0.32 a
Average	0.21 B	0.34 A	0.33 A	
CV (%)	16.83			

Remarks: Means followed by the same uppercase letters in the same row and means followed by the same lowercase letters in the same column are not significantly different according to the LSD test at 5%.

Figure 1. Performance of muskmelon as affected by BOP2 treatment (0 t ha^{-1} Biochar + 50 ml L^{-1} CLF enriched 2 g L^{-1} sodium bicarbonate), and several other samples at 40 days after planting

Figure 2. Performance of muskmelon plants when producing fruit (generative phase)

ate produced the longest plant length compared to when the plants were also given biochar 2-4 t ha^{-1}.

The number of branches also increased significantly when the plants were given CLF, either without sodium bicarbonate enriched or with sodium bicarbonate. There was an effect of sodium bicarbonate on improving the quality of liquid fertilizers and biochar, thereby producing the highest number of branches. A lot of branches will produce high vegetation as well so that it will provide large parts performing high photosynthesis activity. Consequently, higher photosynthates are produced, which will affect the formation of fruit and other organs. The stiver weight of melon plants was the highest when the plants were fertilized with 50 ml L^{-1} of CLF. The effect of the highest dose of biochar (4 t ha^{-1}) was not significantly different from the effect of the dose of 2 t ha^{-1} on the fresh stover weight of melon plants. It turns out that the more branches produced will produce higher fresh stover weight. Plants that get enough nutrients from the application of CLF are producing high fresh stover weight as well.

According to Figure 1, plants grew normally at 40 days after planting because they were still young. Even though the soil pH was around 3-4, the growth of melons was still in the normal category. At that age, necrotic or drought symptoms did not

Table 3. Effects of Biochar and CLF enriched with sodium bicarbonate on the muskmelon yield

Treatments	Provision of CLF + Sodium Bicarbonate			Average
Biochar	0 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 0 g L^{-1}	50 ml L^{-1} + 2 g L^{-1}	
		Fruit circumference (cm)		
0 t ha^{-1}	33.67	41.67	41.33	38.89
2 t ha^{-1}	36.33	40.33	42.17	39.61
4 t ha^{-1}	38.50	39.00	42.17	39.89
Average	36.17 B	40.33 A	41.89 A	
CV (%)	8.23			
		Fruit weight (kg)		
0 t ha^{-1}	0.54 bA	1.15 bA	1.07 bA	0.92
2 t ha^{-1}	0.77 aB	1.30 aA	1.19 aAB	1.09
4 t ha^{-1}	0.82 aA	1.18 bA	1.19 aA	1.06
Average	0.71 B	1.21 A	1.15 A	
CV (%)	6.67			

Remarks: Means followed by the same uppercase letters in the same row and means followed by the same lowercase letters in the same column are not significantly different according to the LSD test at 5%.

appear, indicating the plants got a balanced nutrient. However, when compared with melons planted in soils that have an ideal pH for melons between 6-6.5, the plant height and plant performance were still not optimal (Lena, Jamilah, & Haryoko, 2018).

Figure 3 shows that plants given 4 t ha^{-1} biochar followed by CLF produced leafy leaves that were very healthy without necrotic symptoms. Meanwhile, the application of 4 t ha^{-1} biochar combined with CLF enriched with 2 g L^{-1} sodium bicarbonate showed some necrotic spots. Damage to these plants does not affect the length of the plant or the number of branches of muskmelon plants but will affect the fresh stover weight. In this observation, plants were harvested for the next 10 days. It was proven that the fresh stover weight of the plant treated with BOPO was indeed the lowest compared to the plants with other treatments (Table 2), showing dried and necrotic leaves). This result is due to the fact that plant nutrients provided from the soil and basic fertilizers are inadequate to maintain plant growth and health.

Necrotic symptoms can be caused by plants lacking nutrients or due to pest attacks. The leaves attacked are specifically dominant in the plants that get control treatment (BOPO). However, plants getting good fertilizer treatment from either biochar or CLF produced healthy and fresh green leaves. These healthy leaves are very supportive in photosynthetic activity. Photosynthesis going well will be able to result optimal photosynthates, so as to increase the size and weight of melons.

Fresh stover weight of muskmelon plants increased by 61.90% in plants given CLF compared to the fresh stover weight of plants without CLF. There was no progressive response from plants given CLF with or without sodium bicarbonate. This result showed that sodium bicarbonate was not able to significantly improve nutrient uptake in melon plants.

The circumference and weight of melon fruit were more influenced by the administration of CLF than biochar administration (Figure 2 and Table 3). The enrichment of sodium bicarbonate in CLF was proven to increase the growth and yield of melons but was not significantly different compared to those only given CLF. Fruit circumference increased by 11.5% in plants given CLF compared to the fruits that were not given CLF. The circumference of melons ranged from 33 to 42 cm, similar to what was reported by Lena Ananda Putri, Jamilah, (2018), which was 32-42 cm, with

Figure 3. Plants aged 62 DAP, B0P0 (control) dried from the edge of the leaf, B1P2 (2 t ha^{-1} Biochar + 50 ml L^{-1} CLF enriched 2 g L^{-1} sodium bicarbonate) and B2P1 (4 t ha^{-1} Biochar + 50 ml L^{-1} CLF) looking green without significant brown necrotic

Figure 4. The appearance of muskmelon fruits (left) and muskmelon fruits that have been opened (right)

the highest weight of melons per fruit of 1.33 kg.

There was an interaction effect of the biochar and liquid fertilizer on the weight of the muskmelon fruit (Table 3, Figure 2). The highest fruit weight was 1.30 kg per fruit, observed in plants given 2 t ha^{-1} biochar combined with 50 ml L^{-1} CLF every week, while the lowest fruit weight was observed in plants with control treatment. Jančík, Homolka, Čermák, & Lád (2008) mention that the weight of muskmelons can be up to 2.8 kg per fruit, which means that the muskmelon fruit in this study can still have a chance to increase by up to two times. This result proved that Ultisol was still not optimally able to provide nutrients for muskmelon plants, even though biochar and CLF were also given. The application of biochar and CLF enriched with sodium bicarbonate was proven to improve the growth of muskmelon. The fruit weight of muskmelon given 50 ml L^{-1} CLF increased by 70.42% compared to the fruit weight of muskmelon that were not given liquid fertilizer. This result showed that Ultisol, which was not given both, did not help increase the growth and yield of Muskmelons. From this condition, it is clear that melon cultivation really needs fertile soil.

Compared with the results of the study by Lena Ananda Putri, Jamilah (2018), at the same 50 ml L^{-1} CLF treatment, the yield in this study was still lower at around 0.94 kg per fruit. At the same dose, the fruit weight of the muskmelon was higher when 2 t ha^{-1} biochar was also given, reaching 1.3 kg per fruit. The high fruit weight of melon was also due

to the influence of the vegetation growing better due to higher number of branches (Table 2 and Figure 4). The plants obtained good nutrition, including balanced nitrogen, phosphorus, and potassium. The microelement was also obtained by the plants from CLF because the fertilizer also contains a complete microelement.

CONCLUSIONS

There was a significant interaction effect of biochar and CLF on reducing the soil pH in the rhizosphere of muskmelon plants. Fruit circumference and fruit weight increased by 11.5% and 70.42%, respectively, when given CLF. Among all treatments, the application of 2 $t.Ha^{-1}$ biochar combined with 50 ml of L^{-1} CLF + 0 g L^{-1} sodium bicarbonate resulted in the highest growth and yield of muskmelons, reaching 1.30 kg per fruit per plant.

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Detection and Identification of Polymorphism in Mutant Strawberry (*Fragaria* spp.) Plants Based on *Cleaved Amplified Polymorphic Sequences* Molecular Markers

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ABSTRACT

Strawberry (Fragaria spp.*) has a high economic value and various benefits, but the production of strawberry plants in Indonesia is still low in terms of both quantity and quality. Strawberry plant breeding can be done in various techniques, one of which is polyploidization. Polyploidization by an induction of colchicine at various concentrations in strawberry crops cv. California and Festival resulted in superior phenotype characteristics. To prove the existence of a change in ploidy in strawberry, then research at the molecular level needs to be done. The purposes of this study were to find out changes in ploidy of strawberry plants using CAPS molecular markers and to detect the polymorphism in strawberry plants quickly. The samples used were young leaves. Main procedure was the cutting of the amplified DNA using restriction enzymes of TaqI and HaeIII. The results showed that CAPS molecular markers were capable of detecting polymorphism quickly and efficiently in strawberry plants. Specific bands among strawberry plants having undergone polyploidization and those not having undergone polyploidization can be seen on the differences in monomorphic or polymorphic bands between the control plants and treated plants.*

Keywords: CAPS, Colchicine, Strawberries

ABSTRAK

Stroberi (*Fragaria* spp.) memiliki nilai ekonomi tinggi dan berbagai manfaat, namun produksi tanaman stroberi di Indonesia tergolong rendah baik secara kuantitas maupun kualitas. Pemuliaan tanaman stroberi dapat dilakukan dengan berbagai teknik, salah satunya adalah poliploidisasi. Poliploidisasi dengan induksi kolkisin dalam berbagai konsentrasi tanaman stroberi kultivar Californica dan Festival menunjukkan tanaman stroberi dengan karakteristik fenotip yang unggul. Perubahan ploidi pada tanaman stroberi perlu dibuktikan dengan melakukan penelitian di tingkat molekuler. Tujuan penelitian ini adalah untuk mengetahui perubahan ploidi tanaman stroberi menggunakan marka molekuler CAPS dan mendeteksi polimorfisme secara cepat pada tanaman stroberi. Sampel yang digunakan berupa daun muda stroberi kultivar Californica and Festival. Prosedur utama yang dilakukan pemotongan hasil DNA amplifikasi dengan enzim restriksi *Taq*I dan *Hae*III. Hasil penelitian menunjukkan bahwa penanda molekuler CAPS mampu mendeteksi ploidi dengan cepat dan efisien polimorfisme pada tanaman stroberi. Pita spesifik antara tanaman stroberi yang telah mengalami poliploidisasi dan yang tidak poliploidisasi dapat dilihat pada perbedaan pita monomorfik atau polimorfik antara tanaman kontrol dan tanaman perlakuan.

Kata Kunci: CAPS, Kolkisin, Stroberi

INTRODUCTION

Strawberry plants are widely grown in highland areas in Indonesia. Strawberry plants are grown in some Indonesian areas such as in Lembang and Cianjur areas, West Java province. In addition, strawberry plants are grown in Banyuroto Agritourism area, Banyuroto Village, Sawangan subdistrict, Magelang district, Central Java province. Strawberry plants grow well in this area due to the low temperature that is similar to their natural habitat. Strawberry plant is one of the fruit crops having high economic value and various benefits. Strawberry fruit can be used as food in fresh or processed state. Strawberry fruit also has properties that are good for health (Budiman and Saraswati, 2005).

Strawberry plant production in Indonesia is still low in terms of both quantity and quality. Plant breeding can be done in various techniques, such as hot or cold temperature shock, pressures, and induction using chemicals. One of plant breeding techniques is polyploidization through the induction of colchicine. This study begins with the optimization of the concentration and time of colchicine induction in strawberry (*Fragaria* spp.) plants of California and Festival cultivars. This

field-scale application research regarding the results of the optimization of the colchicine induction in the California and Festival cultivar-strawberry plants was carried out in Banyuroto Village, Sawangan, Magelang district. The research was continued to the cytogenetic level by characterization of chromosomes of strawberry plants cv. California and Festival by Alyza (2015) and Khoiroh (2015). Characterization of phenotype and chromosome alone could not determine the overall character of the strawberry plant. Thus, it is necessary to perform research at the molecular level.

Molecular research has been widely done by using molecular markers such as AFLP, SSR, CAPS Marker, RFLP, and RAPD. The molecular marker used in this research for the detection of polymorphism of the strawberry plant was CAPS (Cleaved Amplified Polymorphic Sequences). CAPS is a PCR based molecular marker. According to Konieczny and Ausubel (1993), the CAPS marker is also called PCR-RFLP marker. CAPS uses the amplified DNA fragments, then the PCR products are cut with restriction enzyme. The advantage of this marker is the inexpensive and simple extraction method that requires a small quantity of DNA template, as well as having codominant nature and specific locus (Matsumoto and Tsumura, 2004). The purpose of this study was to detect polymorphism in the strawberry plants cv. California and Festival to determine the capability of CAPS molecular markers in detecting polymorphism in strawberry plants quickly, as well as to know the difference in the specific bands of California and Festival cultivars.

MATERIALS AND METHODS

The research was conducted from December 2014 to May 2015. Sampling of the strawberry plant leaves was done at Banyuroto Strawberry Agritourism Center, Banyuroto Village, Sawangan Subdistrict, Magelang District. The study was conducted at the Laboratory of Genetics, Faculty of Biology and Laboratory of Genetics and Plant Breeding, Faculty of Agriculture.

The sample used was the young leaves of strawberry plants cv. California and Festival. DNA was extracted using CTAB buffer, phenol: chloroform: isoamyl alcohol (PCIA) with a ratio of 25: 24: 1, 70% ethanol, 100% ethanol, PVP, β-mercaptoethanol, liquid nitrogen, and tissue paper. Master mix, aquabidest sterile (ddH_2O), DNA isolate, and forward and reverse primer were used to amplify DNA. Regarding the research by Kunihisa et al., (2005), the specific primer was APX4 with restriction enzyme of *Taq*I and the primer pair was PYDB with restriction enzyme of *Hae*III. DNA was cut using sterile aquabidest (ddH_2O), DNA amplification, enzyme buffer, and a restriction enzyme. DNA electrophoresis was performed using loading dye, parafilm, agarose gel, TBE buffer and EtBr dye.

The equipment used were microtube with a size of 1.5 ml and 1 ml, vortex, centrifuge, freezer, water bath, analytical balance, pipette tips and micropipette in various sizes, as well as mortar and pestle that had been sterilized. Amplification and incubation of DNA cut was performed with restriction enzyme using thermocycler. Qualitative analysis was done using electroporator, while quantitative analysis was carried outusing a spectrophotometer.

DNA Extraction

Samples were the young leaves of Festival and California cultivars. Each sample used was 0.3 gram that was replicated three times. DNA was extracted from the leaves of strawberries that were already prepared. DNA was isolated using CTAB buffer. The samples (0.3 g) were added with PVP (0.02 g) that were ground finely with liquid nitrogen. Samples were put into microtube of 1.5 mL plus 700 mL CTAB buffer that had been incubated

at a temperature of 65°C for 5 min. Next, the samples already supplemented with CTAB buffer were incubated in a water bath at a temperature of 65°C for 10 min and then incubated at -20°C for 5 min. The sample was then added with 500 mL of phenol-chloroform-isoamyl (24: 1) and inverted for 30 min. Afterwards, it was stored at -20°C for 5 min and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was removed at 200 mL and added with isopropanol at 1x volume of supernatant, gently inverted and then left overnight at 4°C. Afterwards, the sample was centrifuged at 10,000 rpm for 10 min at 4°C. Pellet was collected and washed with absolute ethanol and then centrifuged at 14,000 rpm for 10 min at 4°0C. The second washing was done with 70% ethanol and centrifugation at 14,000 rpm for 10 min at 4°C. Pellet was air-dried until the residual ethanol disappeared, then added with 1X TE buffer plus 50 mL and stored at -20°C.

Qualitative and Quantitative DNA Test

Qualitative test of genomic DNA was done using electrophoresis with an agarose gel concentration of 0.8%. Gel was stained with ethidium bromide (EtBr) for 30 min. Results were observed under UV Transilluminator. The concentration and purity level of the isolated DNA can be identified by DNA quantitative test using a spectrophotometer. The purity of DNA solution can be calculated by comparison of wavelengths of 260 nm to 280 nm. Purity limit commonly used in molecular analysis on the ratio A260/A280 is 1.8 to 2.0. If the value of the ratio is less than 1.8 then there is still protein or phenol contamination in the solution (Sambrook and Russell, 2001).

DNA Amplification

The isolated DNA was amplified using a thermocycler. Specific primers used in this study were the APX-4 and PYDB (Table 1).

Tabel 1. CAPS marker primers used in the study (Kunihisa et al., 2005)

No.	Name of CAPS-RE	Name of Specific Primers	Sequence	Endonuclease
1.	APX4-*Taq*I	APX2-Fw	5′-GTCTCCGATCCCTATCTTTTCTTT- 3′	*Taq*I
		APX2-Rv	5′-TCAGGTCCACCGGTGACC- 3′	
2.	PYDB-*Hae*III	PYDB Fw	5′-AGGTAAGGAACATGATCAACTTTGAG- 3′	*Hae*III
		PYDB-Rv	5′-ATCTGAAAAACCAAGTAGAAACTTACG- 3′	

The amplified DNA was tested qualitatively using electrophoresis with 2% agarose gel. The optimized annealing temperature was 55.1°C.

CAPS-RE Procedures

The amplified DNA was treated with 4U of endonuclease in a 0.01 mL volume. The products of the PCR were cut with restriction enzymes. Restriction enzymes used were *Taq*I for APX-4 primer and *Hae*III for PYDB primer (Kunihisa et al., 2005). First, 3.5 mL sterile aquabidest (ddH_2O) was put into microtube added with 1 mL reaction buffer, 5 mL amplified DNA and 0.5 mL restriction enzyme in each 10 mL reaction. Before the restriction enzyme was put into microtube, the reagent mixture was vortexed for about 5 seconds. Total reaction was 10 mL in 1 mL microtube.

Samples were incubated in a thermocycler (PCR) at a temperature of 65°C for APX4-*Taq*I and at 37°C for PYDB-*Hae*III for 1 hour. After incubation, the samples were subjected to heat inactivation at a temperature of 80°C for 20 min to stop the restriction enzyme action. The results were separated by electrophoresis using an agarose gel 2% in 50 V for 50 min.

RESULTS AND DISCUSSION

The results of DNA amplification using the PYDB primers and cutting of amplified DNA with

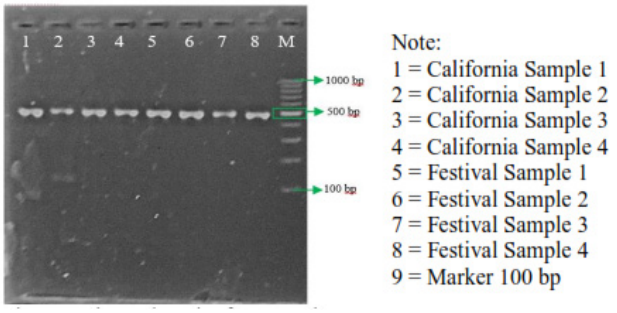

Figure 1. Electrophoresis of PCR products with PYDB primers using 2% agarose gel

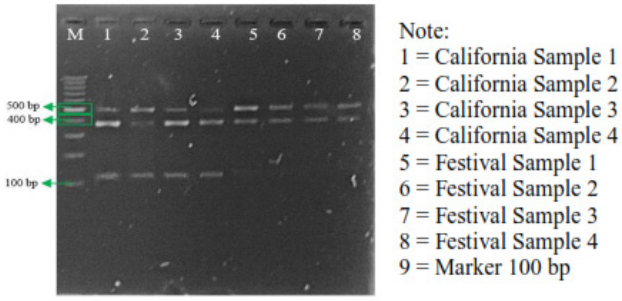

Figure 2. Cutting patterns with HaeIII restriction enzyme

restriction enzyme *Hae*III are shown in Figure 1 and 2. The results of the electrophoresis showed the DNA amplification using PYDB primers with the size of the DNA fragment of 500 bp in all samples of Festival and California cultivars (Figure 1). Figure 2 shows the amplification of DNA that was cut with restriction enzyme of *Hae*III in California sample 3 separated into three DNA fragments with sizes of 500 bp, 400 bp, and 120 bp (Figure 2. No. 1-4). In Festival cultivar, the DNA bands were separated into two fragments with sizes of 500 bp and 400 bp (Figure 2. No. 5-8). Figure 3 shows that all samples were amplified and existing in 400 bp in size both in California and Festival cultivars. Figure 4 represents the electrophoresis results of amplified DNA cutting with enzyme *Taq*I that generates two fragments of DNA in California cultivar with sizes of 400 bp and 300 bp. In Festival cultivars, the amplified DNA that has been restricted by enzyme does not experience separation and DNA remains in the same size with the amplification product of 40 bp.

Several steps taken in this research included DNA isolation of strawberry plants, amplification of DNA with specific primers and DNA amplification product cutting by restriction enzymes. The first step was the isolation of strawberry plant DNA using CTAB (Cetyl Trimethyl Ammonium Bromide) method following the modified protocol of Doyle and Doyle (1990). Isolation of genomic DNA of strawberry plant leaves requires optimization of the procedures and the steps of work because of the high content of secondary metabolites, polyphenols and polysaccharides. Polyphenols and polysaccharides are inhibitors in the process of DNA isolation, which can increase the viscosity of the sample, can be precipitated along with DNA that will degrade the quality of DNA, and may hamper the performance of PCR. The electrophoresis results of genomic DNA (Figure not shown) indicated that the genomic DNA bands were larger than 1 kb, not far from well, and above DNA Ladder.

The composition of the reagents in the reaction included Nuclease Free Water (water), PCR mix, forward and reverse primer, and DNA. This total reaction was enough to do the next step, which was the cutting with a restriction enzyme. The amplified DNA was tested qualitatively by 2% agarose gel electrophoresis. Figure 1 and 3 show the amplified DNA bands with both two primers used. Target amplification size does not differ much from the reference journal, which is in range of 400-500 bp.

Primers used in the study were obtained from reference journals published by Kunihisa et al. (2005), which are specifically designed for CAPS molecular marker. Genome DNA was amplified using two specific primers of APX4 and PYDB. APX4 derived from gene APX (ascorbate peroxidase), while PYDB is single gene, representing a PYD (pyruvate decarboxylase) gene (Kunihisa et al., 2005).

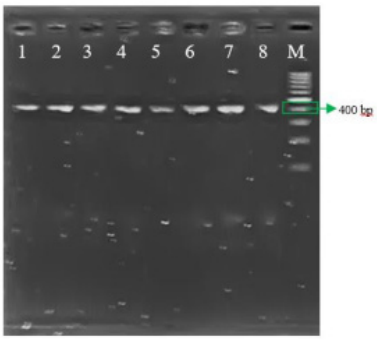

Figure 3. Electrophoresis of PCR products with APX4 primers using 2% agarose gel

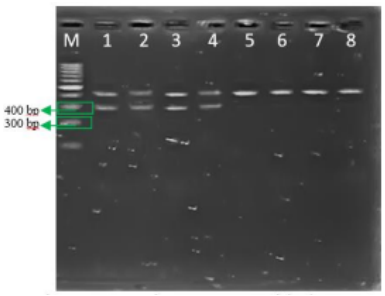

Figure 4. Cutting patterns with the TaqI restriction enzyme

The last step of this research was the cutting of the amplified DNA with a restriction enzyme using CAPS (Cleaved Amplified Polymorphic Sequences) method. CAPS method is a combination of RFLP and PCR methods, but the CAPS is more efficient and simpler because it does not require difficult techniques. The working principle of CAPS molecular marker is that the amplified DNA with specific primers is cut by restriction enzymes and separated by electrophoresis. The results of restriction enzyme cutting will form polymorphic and monomorphic bands, and the differences in these bands can detect genetic differences among individuals.

Restriction enzymes used were *Taq*I and *Hae*III. Enzyme of *Taq*I pairs with APX4 primer, whereas *Hae*III pairs with PYDB primer. Primers are designed specifically to fit the cutting point of restriction enzymes to be used. *Taq*I endonuclease was derived from the bacteria of *Thermus aquaticus* YT I , which has an identifier sequence of T ↓ CGA (Sato, 1978). Meanwhile, *Hae*III endonuclease discovered in 1970 was derived from species Haemophilus *Aegyptus*, which has the identifier sequence of GG ↓ CC (David, 1989; Fatchiyah, 2011).

When the restriction enzyme has recognized its specific sequences, the enzyme will cut phosphodiester bonds into two parts in the backbone of phosphate groups and pentose sugar of DNA double helix. In the restriction enzyme, there is a term "palindromic", which is the same recognition sequences read from the 5 'to 3' of both parts of the DNA double chains. A restriction enzyme fragment produces 3'hydroxyl (OH) group and 5'phosphate (PO_4^-) group. Furthermore, the DNA becomes several fragments corresponding to the cutting area (Hartl and Jones, 1998). *Taq*I restriction enzyme produces fragment with asymmetric ends (sticky ends), whereas the *Hae*III fragment forms blunt ends.

In Figure 2, electrophoresis with primer pairs of PYDB-*Hae*III restriction enzymes showed that in all California samples, DNA bands were separated into three fragments. In the control and all Festival samples, the restriction products were separated into two DNA fragments. Polymorphism between California and Festival cultivars was clearly visible as the California and Festival cultivars showed specific and different DNA bands. Festival cultivar did not show any third DNA fragment in a size of 120 bp.

Separation by electrophoresis (Figure 4) with primer pairs of APX4-*Taq*I restriction enzymes in all California samples showed that they were separated into two DNA fragments. Restriction product on all Festival samples did not show separation, in which there was only one DNA band with a monomorphic band. Polymorphism between the two cultivars were obviously visible as the California cultivar showed a second DNA fragment with a size of 300 bp that was not shared by Festival cultivar.

CONCLUSION

The CAPS molecular markers were capable of detecting polymorphism in the strawberry plants cv. California and Festival quickly and efficiently, which showed that those cultivars had different patterns of DNA band cutting and difference in specific DNA bands. In primers of PYDB-*Hae*III restriction enzymes, California cultivar showed three DNA fragments, while Festival cultivar did not have third DNA fragment that was 120 bp in size. In primers of APX-4 *Taq*I restriction enzymes, California cultivar had two DNA fragments, while Festival cultivar didn't undergo segregation by restriction enzymes and didn't have a second fragment of 300 bp.

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New Promising Rice Genotypes of SP87-1-1-2 and SP73-3-1-7 Adaptive to Lowland and Medium Land

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ABSTRACT

High yield potential (more than 8 ton ha^{-1}), resistant to pest and disease, adaptive to specific or broad environment and good palatability are among rice traits preferred by farmers and consumers. In order to develop such superior rice, yield testing at different agro-climates for some promising lines bred is necessary. This study aimed to examine selected rice genotypes in two different environments, namely Indramayu (9m asl) and Jatinangor (752m asl) and to obtain environment factors affecting the plant trait variation. The experiment was conducted during dry season with fifteen F_5 genotypes, arranged in an augmented design. Based on Least Significant Increase (LSI), genotypes showing better performance than checks were SP87-1-1 on number of productive tillers and total grain weight, SP73-3-1 on panicle length, total grain weight, and weight of 1000 grains. Meanwhile, SP46-4-1 and SP87-4-1 showed better number of filled grains than checks in Indramayu. There was no genotype performing higher number of empty grains than that of the checks. Based on the Principle Component Analysis (PCA), altitude contributed to high variation of plant traits. SP87-1-1 and SP73-3-1 are recommended to be grown in medium and lowland ecosystem because they have high productivity in both environments.

Keywords: Augmented; Biplot; PCA; Yield trial

ABSTRAK

Padi berproduktivitas tinggi (>8 ton ha^{-1}), tahan hama dan penyakit utama, adaptif untuk lingkungan spesifik atau luas, serta memiliki mutu tanak baik merupakan karakter yang diharapkan oleh petani dan konsumen. Guna merakit genotipe superior tersebut, dibutuhkan pengujian galur harapan yang telah dimuliakan sebelumnya pada lokasi dengan kondisi agroklimat yang berbeda. Penelitian bertujuan memperoleh daya hasil genotipe padi pada dua lingkungan yaitu Indramayu (9m dpl) dan Jatinangor (752m dpl) serta memperoleh faktor lingkungan yang memberikan kontribusi besar terhadap variasi karakter padi. Percobaan menggunakan padi F_5 dari 15 genotipe terseleksi menggunakan rancangan augmented. Berdasarkan LSI (*Least Significant Increase*) genotipe yang memiliki nilai lebih baik dari cek yaitu SP87-1-1 untuk karakter jumlah anakan produktif dan berat total; SP73-3-1 untuk karakter panjang malai, bobot gabah isi per tanaman, dan bobot 1000 butir. Pada jumlah gabah isi tidak terdapat genotipe yang lebih baik ceknya. SP46-4-1, dan SP87-4-1 menunjukkan hasil lebih baik dibanding cek pada jumlah gabah isi di Indramayu. Pada jumlah gabah hampa hampir seluruh genotipe tidak lebih baik dari cek. Berdasarkan PCA (*Principle Component Analysis*), ketinggian tempat memberikan kontribusi terbesar terhadap variasi karakter padi. SP87-1-1 dan SP73-3-1 direkomendasikan untuk ditanam pada dataran medium dan rendah karena memiliki produktivitas tinggi pada kedua lingkungan tersebut.

Kata Kunci: Augmented; Biplot; PCA; Komponen hasil

INTRODUCTION

It is estimated that world human population will reach 8 billion by 2030. It is therefore rice production has to be improved by 50% in order to fulfil the demand (Khush and Brar, 2002). Indonesia, as the third largest rice consumer country, has to prepare for this challenge. According to West Java Central Agency on Statistics (2015), within one decade, rice production in West Java Province is much fluctuated, even decreasing from 12.083.162 ton in 2013 to 11.644.899 ton in 2014, then it continued to decline to 11.373.144 ton in 2015. The decline in rice production should be overcome by improving rice productivity and expanding planting area. To improve rice productivity, innovation in genetic improvement of rice should be developed to create new rice plant types with high yielding, resistant to biotic and abiotic stresses, and high grain quality. Meanwhile, expanding planting area for rice cultivation can be done by opening new land and controlling the conversion of agricultural land to non-agricultural use (Syuriani et al., 2013). Expanding planting area can be an option for facing the emerging issues, such as the increasing human population and climate change.

In addition, high yield and resistance to major pest and disease are main focus of rice breeding as strategic commodity for the development throughout Indonesia with diverse agro-climatic conditions. In order to improve rice productivity, the breeding progress with yield component as a main target (Akinwale et al., 2011) can be seen through yield trial. Yield testing for promising rice lines on various locations with different agro-climatic condition is highly needed since there is a genotype by environment interaction, especially for quantitative traits.

Environment factors contribute to genotype performance. One of the responses of rice plants to the environment is shown by the level of adaptability. Plants are classified to be adaptive if they are able to show good yield on various environmental conditions (Annicchiarico, 2002). Some rice cultivars have been known to have different adaptability in specific or broad location. For example, some rice lines as found by Dianawati and Noviana (2015) are adaptive to low land ecosystem. Meanwhile, Caredek Putih and Caredek Merah, local varieties grown in West Sumatera (Syarif and Zen, 2012), are adaptive to high altitude. Other cultivar such as IR64 is well known as high-yielding mega-variety that has a broad adaptation (Mackill and Kush, 2018). These data suggest that genetic composition of rice, especially genes that controlling adaptation ability, play significant role in determining adaptability. Information regarding on environmental factors of two research locations are interesting to be elaborated. In addition, two diverse locations will give more information regarding to the environmental factors mostly contributing to the rice variations observed that will be analyzed by PCA (Principal Component Analysis; Pearson, 1901).

Yield trial has been done previously for some rice genotypes, yet it could not provide information on the specific environmental factors that contribute to the variation of rice traits. In this experiment, specific environment factors that contribute to the variation were comprehensively analyzed, making it different from some experiments that have been done (Uzzaman et al., 2015, Barma et al., 2018, Abebrese et al., 2019). Therefore, the objectives of the experiment were to obtain yield potential of promising improved rice lines in two diverse locations and to determine any specific environments that contribute significantly to the variation of observed rice traits.

MATERIALS AND METHODS

Experiment Location

The experiment was carried out from June to November 2016 in two sites with different growing condition, which were Karangampel (Indramayu) and Jatinangor (Sumedang). Both locations were chosen based on the difference in altitude and agro-climate. The description of both sites is provided in the Background. Indramayu has an altitude of 9 m above sea level (asl), coastal area, climatic type tropical warm with a temperature of 22.9 - 30°C, humidity of 70-90%, latitude of -6.49465 and longitude of 108.4244, climatic type D according to Schmidt and Ferguson, and is one of national rice production centers. Meanwhile, Jatinangor has an altitude of ±753m asl with latitude of -6.91752 and longitude of 107.7704, and climatic type C according to Schmidt and Ferguson (Kementerian Pertanian, 2015b).

Plant Materials

Seeds of 15 new promising rice lines (F5) derived from previous molecular and phenotypic selection and eight check cultivars were used. The checks were Pandanwangi, Sintanur, IR-64, PTB-33 (parents) and some widely grown cultivars, i.e. IR-42, Inpari-13, Ciherang, and Kebo (local variety).

Experimental Design and Data Analysis

The experiment was arranged in augmented design that is suitable with limited number of seeds available. Sharma (2006) pointed out that augmented design applied for inappropriate land condition and number of genetic materials tested. Variables measured were yield and its component, including number of productive tillers, panicle length, number of filled graons, number of empty grains, weight of 1000 gains, and total grain weight per plant. Environmental elements observed were latitude (LT), longitude (LG), altitude (HG), maximum temperature (TMAX), minimum temperature (TMIN), average temperature (TAVG), rainfall (RN), relative humidity (RH), photoperiod (SUN), maximum wind speed (MAXWD), average air pressure (AVGUST), and average wind speed (AVGWD). Data normality was tested by using Kolmogorov-Smirnov Z, while Bartlett's test was used for homogeneity of variance and then continued to combined analysis of variances. Comparison of agronomic performance and yield of new promising genotypes with those of the checks on the two sites was performed by LSI (Least Significant Increase). Estimation of environmental contribution to the variation of rice traits was conducted by PCA (Principal Component Analysis).

RESULTS AND DISCUSSION

Preliminary yield testing for some crops has been practiced by breeders since some years ago in order to evaluate promising genotypes under different locations or environments. This study presented preliminary yield trial of 15 new promising rice lines in two locations. A combined analysis of variance for yield and yield component traits to obtain genotype by environment interaction was performed. Petersen (1994) mentioned that genotype by environment interaction illustrated whether the genotypes tested were uniform in different environments. Genotypes that performed constant superior traits in all locations could be developed into widely adapted varieties, and then they could be released (Ganefianti et al., 2009). In this experiment, normality test found that the data were normally distributed, and the variance was homogenous, thus the data were valid for a combined ANOVA as presented in Table 1.

Table 1. A combined ANOVA analysis for yield and yield component on two locations

No.	Traits	CV (%)	Mean Square GXE	F value GXE
1.	Number of productive tillers	11	30.52	8.74*
2.	Length of panicle	4	12.97	14.41*
3.	Number of filled grains	3	47584.49	23.15*
4.	Number of empty grains	9	9053.87	3.56*
5.	Weight of 1000 grains	2	17.94	81.55*
6.	Total grain weight per plant	1	110.01	250.02*

Remarks: * significantly different at $p < 0.05$ based on combined ANOVA; CV= Coefficient of Variation; GxE= Genotype by Environment Interaction.

Referring to Table 1, it was found that there was genotype by environment interaction effect on six traits in both locations. The genotypes tested differed in their performance in both locations. It also indicated that the performance of traits was affected by genotype by environment interaction. Fehr (1987) stated that the influence of the environment on genotype by environment interaction was often related to location and season factors. Variation on environmental factors such as temperature, relative humidity, rain fall, photoperiod, light intensity, soil fertility, and other factors could contribute to the interaction phenomenon affecting rice yield. Significant performance of yield and yield component traits exhibited the variability of rice traits in both planting locations.

Based on Table 1, the range of the Cofficient of Variation (CV) was around 1-11%. It implies that the data are good since the CV value is less than 20% as stated by Gasperz (2006), and the traits

observed have a relatively high level of confidence. The greater the CV, the greater the significance level of the study conducted. It also reveals that the data collection was valid and well conducted.

Yield potential of 15 Promising Rice Genotypes

Improving yield potential of rice is an important objective for almost all rice researchers (Gravois and Helms, 1992). Rice genotypes as genetic material used in this experiment had reached F5 generation with unknown yield potential. Previous phenotypic and molecular selection for aromatic trait and resistance to brown plant hopper has been made. Assessing yield potential was one of the objectives of this experiment. Yield in rice was dominated by three traits, namely the number of tillers per plant, number of filled grain, and grain weight (Xue et al., 2008). These three traits were already included in this experiment as main variables.

Number of productive tillers and length of panicle

The genotypes of rice grown in Indramayu showed good response on the number of productive tillers (Table 2). Three genotypes of SP87-30-1, SP87-4-1, and SP87-1-1 were better in the number of productive tillers compared to that of the

Table 2. LSI test for number of productive tillers and length of panicle in Indramayu and (Jatinangor) Sumedang

No.	Genotype	Number of productive tillers				Length of panicle (cm)			
		Indramayu		Jatinangor (Sumedang)		Indramayu		Jatinangor (Sumedang)	
		Value	Notation	Value	Notation	Value	Notation	Value	Notation
1.	IP158-5-1	18.07	fh	16.68	bcfgh	21.36		21.65	e
2.	PP48-3-1	15.43	f	17.32	bcfgh	23.8	bdfgh	20.48	
3.	SP101-3-1	17.43	fh	17.52	bcfgh	22.46	gh	21.08	e
4.	SP46-4-1	22.31	adefgh	22.83	bcdefgh	21.57		19.57	
5.	SP73-1-1	19.33	dfgh	22.08	bcdefgh	21.61		22.95	eh
6.	SP73-3-1	18.82	fgh	17.21	bcfgh	26.12	abcdgh	24.18	e
7.	SP87-10-1	18.71	fgh	19.73	bcdefgh	19.89		21.35	e
8.	SP87-1-1	25.62	abcdefgh	23.08	bcdefgh	23.13	fgh	21.56	e
9.	SP87-15-1	16.11	fh	12.9	gh	21.32		22.02	e
10.	SP87-24-1	15.96	f	12.29	gh	22.02		22.79	e
11.	SP87-25-1	19.24	fgh	15.29	fgh	21.14		23.12	e
12.	SP87-26-1	23.25	adefgh	12.74	gh	23.35	efgh	19.78	
13.	SP87-27-1	23.15	adefgh	17.89	bcfgh	21.53		28.03	bcdefh
14.	SP87-30-1	26.56	abcdefgh	16.63	bcfgh	21.81		20.63	
15.	SP87-4-1	30.89	abcdefgh	13.64	fgh	22.41	gh	20.62	
16.	PTB-33 (a)	20.08		23.32		24.59		28.11	
17.	IR-42 (b)	23.32		15.65		23.61		24.28	
18.	Kebo (c)	23.65		15.98		24.11		25.28	
19.	Pandanwangi (d)	19.32		19.32		23.28		23.45	
20.	IR-64 (e)	20.09		18.03		27.24		20.90	
21.	Sintanur (f)	12.92		12.96		22.97		25.02	
22.	Inpari-13 (g)	18.61		9.81		22.23		24.03	
23.	Ciherang (h)	11.98		11.68		22.41		22.84	

Remarks: LSI (Least Significant Increase) value for number of productive tillers = 0.647569; panicle length= 0.275346; Alphabet a, b, c, d, f, g, h means that that the tested genotype is significantly different compared to the checks listed as follow: a = PTB-33, b = IR-42, c = Kebo, d = Pandanwangi, e = IR-64, f = Sintanur, g = Inpari, h = Ciherang according to LSI at 5%.

checks (PTB-33, IR-42, Kebo, Pandanwangi, IR-64, Sintanur, Inpari-13, Ciherang). Meanwhile, in Jatinangor, there were no genotypes performing better than the seven checks (IR-42, Kebo, Pandanwangi, IR-64, Sintanur, Inpari-13, Ciherang), except four genotypes of SP46-4-1, SP73-1-1, SP87-10-1, and SP87-1-1. Number of productive tillers is important trait that contributes to rice yield. Tiller development in rice is affected by many factors, including organic materials in soils, planting distance, and weather conditions such as light intensity, temperature, and water supply (Yan et.al., 1998). According to LSI, the genotype of SP87-1-1 tended to be better than the checks in the two locations. Number of tillers is a quantitative trait inherited from the parents (Xiong, 1992). Number of productive tillers of PTB-33 was greater than that of Sintanur in both locations. However, the genotype of SP87-1-1, progeny of PTB-33 and Sintanur, had productive tillers that was much higher than their parents. It is likely that number of productive tillers from both parents is successfully inherited. Genetic composition in terms of allelic composition of SP87-1-1 is favorable in controlling number of productive tillers. This character will develop more optimally along with the decrease in the number of unproductive tillers. Sheehy et al. (2001) pointed out that one of the goals of rice breeding in the terms of agronomy in existing cultivars was to minimize the number of unproductive tillers. Unproductive tillers do not contribute to the increasing Leaf Area Index (LAI) and yield. An increase in the number of productive tillers can be optimized by increasing the organic matter, photoperiod, and irrigation (Yan et al., 1998).

Based on LSI (Table 2), SP73-3-1 had significantly longer panicle length than that of the six checks (PTB-33, IR-42, Kebo, Pandanwangi, Inpari-13, Ciherang). SP87-27-1 had significant longer panicle length with those of the checks (IR-42, Kebo, Pandanwangi, IR-64, Sintanur, Ciherang). Panicle is composed by many spikelets and rachillae comprising peduncle and primary, secondary, and tertiary branches. Ando et al. (2008) argued that optimization of panicle balance with its components is needed to increase the number of spikelets directly determining the increase in rice yield. In general, high yielding variety from indica subspecies is tended to have many spikelets than japonica due to longer panicle and stem and higher number of primary and secondary stems. Genotypes used in the experiment are derived from hybridization of cultivars that belong to indica subspecies. Fifteen promising genotypes used in this experiment are predicted to have higher yield than japonica.

The yield of rice plants manifested by the total amount of grain harvested can be divided into two components, namely sink size (the potential capacity for maximum production of crop) and source size (the potential capacity to utilize the photosynthetic products). The source-sink relationship in plants affects the rate of crop yield that is influenced by environmental conditions (Smith et al., 2018). Genotype of SP73-3-1 had better tendency than some checks at in the two planting locations, but it could not exceed panicle length of both parents (Table 1).

Panicle length of the progenies, namely SP (Sintanur xPTB-33), IP (IR-64 x PTB-33), and PP (Pandan Wangi x PTB-33), was not exceeding that of their parents, and this trait is a quantitative trait with low heritability (Mohamed et al., 1965). It is possible if the offsprings do not have traits like the parents nor the combination of the characters of the two parents because this character is difficult to inherit. Panicle development is affected by many environmental factors (Yan et al., 1998). Baker et al. (1992) found that the optimum temperature for rice cultivation was around 22-28°C. The average temperature in Indramayu and Jatinangor planting

were 32.5°C and 28.5°C, respectively. The temperature in Indramayu is not in accordance with the optimum temperature criteria according to Baker et al. (1992). This lack of optimum temperature affected the panicle length in Indramayu, which was not better than in Jatinangor

Number of filled and empty grains

Filled grain is one of the characters that determine the rice yield. The data from LSI analysis showed an interesting phenomenon because of the contrast of the analysis results in Jatinangor (Table 3). Based on LSI analysis, there was no genotype performing higher number of filled grains compared to the checks in Jatinangor. Meanwhile, in Indramayu, there were nine genotypes that were better than several checks, and there was one genotype that was better than all checks (PTB-33, IR-42, Kebo, Pandanwangi, IR-64, Sintanur, Inpari-13, Ciherang), which was SP87-27-1.SP87-24-1 was the most superior genotypes among other genotypes grown in Indramayu because it had a lower number of empty grains than that of five checks (IR-42, IR-64, Sintanur, Inpari-13, and Ciherang) (Table 3). The genotype of IP158-5-1 became the most superior genotype in Jatinangor with lower

Table 3. LSI test for number of filled grains and number of empty grains of rice grown in Indramayu dan Jatinangor (Sumedang)

No.	Genotype	Number of filled grains				Number of empty grains			
		Indramayu		Jatinangor (Sumedang)		Indramayu		Jatinangor (Sumedang)	
		Value	Notation	Value	Notation	Value	Notation	Value	Notation
1.	IP158-5-1	1407.6	e	459.84		844.14	e	380.46	aefgh
2.	PP48-3-1	605.66		766.26		738.94	efh	1036.87	
3.	SP101-3-1	806.98		689.25		671.47	efh	454.72	efgh
4.	SP46-4-1	1641.14	efgh	903.83		771.43	efh	951.08	
5.	SP73-1-1	1331.69	e	897.93		820.26	ef	553.80	efh
6.	SP73-3-1	1613.22	fgh	1342.61		696.41	efh	835.03	h
7.	SP87-10-1	854.58		477.44		1349.71		681.76	aefh
8.	SP87-1-1	1830.16	fgh	1360.74		796.30	ef	850.29	
9.	SP87-15-1	743.30		671.97		1169.97		436.21	aefh
10.	SP87-24-1	1058.22		532.53		424.82	befgh	530.74	efgh
11.	SP87-25-1	799.06		526.69		1036.18		716.03	efgh
12.	SP87-26-1	1277.26	e	731.63		1362.09		668.29	efgh
13.	SP87-27-1	2450.85	abcdefh	517.59		956.95		870.68	
14.	SP87-30-1	1144.69	e	502.08		1025.56		526.08	efgh
15.	SP87-4-1	1735.74	e	372.67		1162.81		518.44	efgh
16.	PTB-33 (a)	2013.40		1876.21		360.75		451.66	
17.	IR-42 (b)	1922.21		1973.21		444.66		319.66	
18.	Kebo (c)	2239.54		2133.54		371.99		349.99	
19.	Pandanwangi (d)	1944.87		2004.21		248.32		338.99	
20.	IR-64 (e)	1071.87		1482.54		865.99		798.32	
21.	Sintanur (f)	1432.87		1415.87		824.99		803.32	
22.	Inpari-13 (g)	1424.21		1648.87		587.99		551.32	
23.	Ciherang (h)	1485.87		1542.54		785.32		843.66	

Remarks: LSI (Least Significant Increase) value for number of productive tillers = 0.647569; panicle length= 0.275346; Alphabet a, b, c, d, f, g, h means that that the tested genotype is significantly different compared to the checks listed as follow: a = PTB-33, b = IR-42, c = Kebo, d = Pandanwangi, e = IR-64, f = Sintanur, g = Inpari, h = Ciherang according to LSI at 5%.

number of empty grains than that of five checks (PTB-33, IR-64, Sintanur, Inpari-13, and Ciherang) in the Jatinangor (Table 3). The number of grains per unit area determined the yield of rice. However, the number of spikelets per unit area is also a major determinant of yield on cereal crops without environmental stress factors (Fischer, 1983; Takeda, 1984; Kropff et al., 1994).

SP73-3-1 (Fig. 1a) becomes the genotype with optimal panicle length, which is expected to produce the optimal number of grains. However, the number of filled grains of this genotype did not exceed that of the checks, resulting in higher number of empty grains exceeding that of the checks. These conditions happened in both planting locations, illustrating the existence of other factors that cause the grains to be not fully filled.

During the rice ripening period, temperature becomes a major factor that influences the duration of grain filling process (Yoshida and Parao, 1977). Matsui et al. (1997) mentioned that rice plants exposed to high temperatures would promote higher spikelet sterility. High temperatures above 300C will induce sterility in the spikelet, disrupt pollination, and reduce yield. The average temperature in Jatinangor was below 30°C, but the average temperature in Indramayu was above 30°C. However, in fact, the number of filled grains in Indramayu was higher than in Jatinangor . It seems that the genotypes tested are more suitable for warm temperature.

The number of grains can be improved by increasing the number of panicles. Modern rice varieties with high yield have a higher number of grains than previous varieties, but there are limits in increasing the number of panicles. Additional tillers that continue to grow can become unproductive so that they only develop more in the vegetative phase, and when producing grains, they are unable to develop filled grains (Khush, 1995). This reference supports that in this experiment, there was no maximal development during vegetative phase, so that the rice grains could not be filled despite the high potential of panicle length. Increasing the number of panicles, panicle sizes, and both can increase the rice yield (sink size). An increase in just one of these characters will not give significant improvement of yield (Ying et al., 1998).

Increased sink size must be accompanied by an improvement in source size. During the filling period of the rice grain, the photoperiod in the planting locations decreased and rainfall increased. However, due to this condition, grain filling process was not supported by optimum photosynthesis process, so that the filling of grains as well as the grains maturation took longer than usual. In short, the generative phase was not optimum, thereby affecting the harvesting time in Jatinangor . Based on the LSI presented in Table 3, there were nine genotypes performing higher number of filled grains than that of the checks in Indramayu, while in Jatinangor , there were no genotypes

Weight of 1000 grains and total grain weight

The weight of 1000 grains weight was observed at 14% water content. In addition, the estimation of the weight of 1000 grains was carried out through calculations based on the guideline from International Rice Research Institute (IRRI, 2009). The contrast data were found at the two planting locations (Table 4). In Indramayu, there was one genotype showing a greater value compared to seven checks, namely SP87-15-1, and in Jatinangor, there was one genotype, namely SP73-1-1, which was better than four checks. The genotypes of SP73-3-1 (Fig. 1b) and SP87-1-1 (Fig. 1b) tended to be better than several checks at the two planting locations on the total grains weight.

SP87-15-1 was the best genotype among other genotypes on the weight of 1000 grains in Indra-

Table 4. LSI test for the weight of 1000 grains and total grain weight in Indramayu dan (Jatinangor) Sumedang

No.	Genotype	Number of filled grains				Number of empty grains			
		Indramayu		Jatinangor (Sumedang)		Indramayu		Jatinangor (Sumedang)	
		Value	Notation	Value	Notation	Value	Notation	Value	Notation
1.	IP158-5-1	30.14	efgh	24.42		37.69	efgh	9.94	
2.	PP48-3-1	28.34	efgh	23.29		14.32		14.69	
3.	SP101-3-1	29.72	efgh	28.47	efh	20.34		16.75	
4.	SP46-4-1	28.36	efgh	21.69		73.65	abcdefgh	19.66	
5.	SP73-1-1	32.69	efgh	30.78	efgh	35.96	efgh	24.62	h
6.	SP73-3-1	26.63	fgh	26.99	eh	32.07	eg	33.26	efh
7.	SP87-10-1	27.69	fgh	26.42	eh	20.32		11.52	
8.	SP87-1-1	30.18	efgh	28.56	egh	42.92	efgh	33.93	efh
9.	SP87-15-1	35.37	abcefgh	22.81		20.36		14.75	
10.	SP87-24-1	30.01	efgh	26.02	eh	25.58		12.44	
11.	SP87-25-1	29.78	efgh	27.01	eh	20.78		12.68	
12.	SP87-26-1	29.63	efgh	23.68		32.97	efg	15.38	
13.	SP87-27-1	30.16	efgh	24.43		68.45	abcdefgh	11.98	
14.	SP87-30-1	28.75	efgh	26.75	eh	27.35	e	12.26	
15.	SP87-4-1	30.75	efgh	23.52		40.55	efgh	8.83	
16.	PTB-33 (a)	35.11		34.74		60.61		64.75	
17.	IR-42 (b)	32.94		34.22		64.75		66.27	
18.	Kebo (c)	34.37		36.73		66.27		50.96	
19.	Pandanwangi (d)	36.45		36.36		50.96		60.29	
20.	IR-64 (e)	27.93		25.14		60.29		67.28	
21.	Sintanur (f)	25.09		27.42		67.28		56.50	
22.	Inpari-13 (g)	20.00		28.93		56.50		58.22	
23.	Ciherang (h)	25.79		25.69		58.22		26.31	

Remarks: LSI (Least Significant Increase) value for number of productive tillers = 0.647569; panicle length= 0.275346; Alphabet a, b, c, d, f, g, h means that that the tested genotype is significantly different compared to the checks listed as follow: a = PTB-33, b = IR-42, c = Kebo, d = Pandanwangi, e = IR-64, f = Sintanur, g = Inpari, h = Ciherang according to LSI at 5%.

mayu because this genotype was better than all checks except Pandanwangi. In Jatinangor, there were only eight genotypes that were better than a few checks. The genotype of SP73-1-1 was the best genotype with higher weight of 1000 grains than that of the four checks (IR-64, Sintanur, Inpari-13 and Ciherang). Based on LSI test, there were two genotypes that were better than all checks on the total grain weight at Indramayu, namely SP46-4-1 and SP87-27-1. Jatinangor planting location showed unacceptable performance for the total grain weight. No genotypes were better than checks. Two genotypes were better than IR-64, Sintanur, Ciherang, namely the SP-73-3-1 and SP-87-1-1. SP73-1-1 was only better than cv. Ciherang. Genotypes of SP73-3-1 and SP87-1-1 tended to be better than several checks at the two planting sites (Table 3).

Grain size is an important quality trait in rice plants (Unnevehr et al., 1992; Juliano et al., 1993) and the major determinant of grain weight. The main components of grain size were number of panicles per plant, number of grains per panicle, and grain weight. At present, the weight of 1000 grains in commercial varieties ranges between 25-35g (Zhang et al., 2012). Genotypes of SP73-1-1, SP101-3-1, SP87-10-1, SP87-30-1, SP87-24-1, SP87-25-1, SP73-3-1, and SP87-1-1 had higher weight of 1000 grains than some checks in both locations

(Table 3). SP46-4-1 and SP87-27-1 were superior in total grain weight as seen in Table 3. Grain size is a trait inherited quantitatively from parents (McKenzie and Rutger, 1983). It indicates that the weight of 1000 grains and total grain weight traits have been inherited in these promising lines.The amount of grain production per unit area of land is the main determinant of yield on cereal crops that grow in high-yielding areas without stress factors (Takeda, 1984). One assessment of yield is the total grain weight. The total grain weight is influenced by the yield components and other environmental factors. One environmental factor that affects total grain weight is temperature. Baker et al. (1992) reported that an increase in the maximum temperature of day or night minimum of 28/21 to 34/27°C reduced the rice yield by 7-8%. In two planting locations, there was no increase in the maximum temperature of the day or the minimum night, which reached 6°C. In general, changes in global climate conditions can result in an increase in air temperatures of 1.4-5.8°C (IPCC, 2001), and according to Peng et al. (2004), there was an increase in average annual minimum and maximum temperatures, which was 0.35°C and 1.13°C, respectively, at IRRI Manila, Philippines. Every temperature increases by 1°C of the minimum temperature in the planting season, yield of rice plants decrease by 10%. This statement was conveyed by Peng et al. (2004) who conducted the experiment. According to this study, there was an increase in temperature at both planting locations up to 1°C. It can be predicted that the total grain weight in the high yielding genotype will be high if the minimum temperature at the planting site is normal. Plant breeders need to increase grain weight per plant by improving the yield component traits (Akinwale et al., 2011). Genetic improvement of rice trait can be focused on the yield component traits that will affect the grain weight and the weight of1000 grains. Genotypes showing superior traits of the weight of 1000 grains and total grain weight can be recommended for further development or to be released as new rice variety.

Contribution of environment factors to the variation of plant traits

The contribution of environmental factors to variations of genotypes' traits grown in Indramayu and Jatinangor can be determined through principal component analysis (PCA). There are two main factors or components (PCs) involved, each factor representing the variables analyzed (Table 5). The eigen value was sorted from the largest to the smallest value, in which the eigenvalue value <1 was not used in calculating the number of factors formed (Jolliffe, 2002). Eigenvalue also shows the relative importance of each factor to estimate the variability of the observed variables (Soemartini, 2008). Based on Table 6, only one factor was formed, and the eigenvalue value has a value of more than 1. Factor 1 (PC1) has an eigenvalue value of 1.808. The eigenvalue means that this factor can explain 1.808 or 90.410% of the total.

Table 5. Eigenvalue and variability in the environmental factors

PC	Eigenvalue	Variability %	% Cumulative
1	1.808	90.410	90.410
2	0.192	9.590	100.000

Table 6. Matrix vector value for environmental factors

Variables	PC1	PC2
Latitude (LT)	-0.710	0.122
Longitude (LG)	-0.253	0.080
Height of place/altitude (HG)	1.054	-1.586
Maximum temperature (TMAX)	-0.553	0.114
Minimum temperature (TMIN)	-0.577	0.120
Average temperature (TAVG)	-0.564	0.116
Rain fall (RN)	0.289	-0.473
Relative humidity (RH)	-0.372	0.056
Photoperiod (SUN)	-0.269	0.132
Maximum wind speed (MAXWD)	-0.647	0.125
Average air pressure (AVGUST)	-0.653	0.128
Average wind speed (AVGWD)	-0.661	0.126

Remarks: Bolded notation is the variable affecting the variation of genotypes' traits with discriminant value >0.5 (Jolliffe, 2002).

Figure 1a. Grains of SP-73-3-1-2

Figure 1b. Grains of SP-87-1-1-7

The height of the location or altitude (above sea level) contributes to variations in the planting location with a variable value greater than 0.5 (Table 6). Based on Table 6, there were two factors (PC) formed. However, the optimal factor to reduce the observed variables is found in PC1 because, in PC1, it can already be seen what variables contribute greatly to the variations that arise at the planting locations. Determination of the variables that contribute was done by looking at the value of each variable in PC1. Variables with a value of more than 0.5 have a large contribution to the variation at the planting location.

Altitude had a greater effect to other environmental conditions. Environmental factors that could be significantly different due to the altitude were temperature and humidity. The rice planting environment is mostly in areas with temperature close to the optimum temperature for rice plant growth at 28/22°C (Baker et al., 1992). Based on the climatological data, the average temperature in Indramayu during the study was around 32°C, with maximum and minimum temperatures of 35°C and 30°C, respectively, while the average temperature in Jatinangor was around 28.3°C, with maximum and minimum temperatures of 3°C and 24°C, respectively. According to Satake and Yoshida (1978), rice plants will be more sensitive to high temperatures and will experience interference at temperatures above 33°C. Disturbances that occur can directly affect rice yield because they occur during the reproductive process of the reproductive organs. Drought that hit rice plants in vegetative phase can reduce total number of grains (Hariyono, 2014)

The average humidity in Indramayu during the study ranged from 69% to the highest of 71%, while in Jatinangor, the average humidity was 16% higher than the average humidity in Indramayu, with the highest humidity of 87%. Humidity of the planting environment will affect the spikelet fertility. The higher percentage of air humidity will decrease fertility of spikelets (Nishiyama et al., 1981, Matsui et al., 1997). Sterility during flowering of rice plants can be induced by high temperature at day and night temperatures of 35°C and 30°C, respectively, together with high humidity of 90% (Abeysiriwardena et al., 2002). The average rainfall in Indramayu was lower than int Jatinangor. During growing phase, the average rainfall in Indramayu was 105.56mm, while in Jatinangor, it ranged at an average of 359.88mm. The high amount of rainfall occurred at the end of the growing season in both locations with successive increase in both Indramayu and Jatinangor reaching 170.51mm and 652.48mm, respectively. In contrast to rainfall, the average photoperiod in Indramayu was longer than in Jatinangor, which was longer by 26.507 hours. The longest photoperiod occurred with 118.66 hours in Indramayu when entering the final stage of maturity and 123.30 hours in Jatinangor in the vegetative growth phase of planting.

Other important environmental factors affecting the rice growth and development is wind. The wind mostly helps in the occurrence of pollination. Wind speed can reduce humidity on anther and spikelet. Matsui et al. (1997) reported that the effect of wind disturbances on pollination might depend on the position of the anther. High wind speed could eliminate a number of pollen and disrupt the self-pollination. Spikelets that develop at high temperatures will be more sensitive to the presence of wind. During planting, the wind speed in Indramayu planting location was at an average of 8.35 miles per hour (mph), whereas in Jatinangor, it was 3.4 mph. The highest wind speed occurred

in Indramayu was 10.1 mph in the third month, while in Jatinangor, it was only 3.6 mph in the second and third months.

CONCLUSION

The genotypes of SP73-3-1 and SP87-1-1 had better yield and yield components than those of the checks in two different planting locations, which were Indramayu (9asl) and Jatinangor (753asl). SP87-1-1 had a higher number of filled grains than the check in Indramayu. The planting location has a contrasting altitude. The height of the place (altitude) is an environmental factor that have largest contribution to the variation of the rice plant traits. The genotypes of SP73-3-1 and SP87-1-1 were able to demonstrate their superiority by being able to adapt and show better yield and yield components than checks at two planting sites. The genotypes of SP73-3-1 and SP87-1-1 are recommended to be grown in low and medium agro-climatic environments.

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Effects of *Chromolaena odorata* Compost on Soil and Nutrient Uptake of Lettuce (*Lactuca sativa*)

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ABSTRACT

The use of synthetic inorganic fertilizers containing chemical compounds cause soil quality to decrease. Chromolaena odorata are potential weeds used as a source of organic matter, which can be used as compost. This research aimed to determine the effect of Chromolaena odorata compost on the soil and nutrient uptake of lettuce. The research was conducted using a single factor experimental method arranged in a Randomized Complete Block Design. The treatments tested were the applications of C. odorata weed compost at various doses (222 grams/pot, 444 grams/pot, and 666 grams/pot) with control treatments of 200 ml/pot NPK Phonska (15:15:15), 320 grams/pot cow manure, and without fertilization. The experiment consisted of three blocks with three samples and three units of sample plants within each treatment. The results showed that the application of C. odorata weed compost significantly improved nutrition and nutrient uptake of lettuce. The dose of 444 grams/pot C. odorata weed compost was the best dose to increase soil quality and nutrient uptake of lettuce.

Keywords: C. odorata, Inorganic fertilizer, Lettuce

ABSTRAK

Penggunaan pupuk anorganik mengandung senyawa kimia menyebabkan kesuburan tanah menjadi berkurang. Gulma kirinyu merupakan gulma yang cukup potensial untuk dimanfaatkan sebagai sumber bahan organik, salah satunya dapat dijadikan sebagai kompos. Penelitian ini bertujuan untuk mengetahui pengaruh dan mendapatkan takaran kompos gulma kirinyu yang terbaik dalam meningkatkan kualitas tanah dan serapan hara tanaman selada. Penelitian dilakukan menggunakan metode percobaan faktor tunggal disusun dalam Rancangan Acak Kelompok Lengkap dengan perlakuan yang diujikan adalah kompos gulma kirinyu dengan takaran 222 gram/pot, 444 gram/pot dan 666 gram/pot, serta perlakuan pembanding yaitu perlakuan takaran NPK Phonska (15:15:15) 200 ml/pot, takaran pupuk kandang sapi 320 gram/pot dan tanpa pemupukan. Setiap perlakuan terdapat tiga blok sebagai ulangan dengan masing – masing perlakuan terdiri dari tiga sampel dan setiap perlakuan terdiri atas tiga unit tanaman korban. Hasil penelitian menunjukkan bahwa kompos gulma kirinyu meningkatkan nutrisi dan serapan hara pada tanaman selada. Takaran kompos gulma kirinyu 444 gram/pot merupakan takaran terbaik dalam meningkatkan kualitas tanah dan serapan hara pada tanaman selada.

Kata Kunci: Gulma kirinyu; Pupuk anorganik; Selada

INTRODUCTION

Lettuce is one of the popular vegetables due to its color, texture, and taste liked by most people (Lutfi, 2017). Besides, lettuce contains nutrients that are beneficial to human body, including vitamins, protein, carbohydrates, calcium, phosphorus, and iron. Lettuce also provides a high economic value as the number of international hotels and restaurants serving dishes such as salads and hamburgers. This is indicated by the increase in demand for lettuce in the world market, amounting to 2,792 tons in 2012 and the large quantity of imported lettuce in 2012, which was 145 tons (Akhlaq, 2018). Therefore, lettuce production needs to be improved. BPS-Statistics Indonesia (2014) showed that the production of lettuce in Indonesia from 2010 to 2013 was 283,770 tons, 280,969 tons, 294,934 tons, and 300,961 tons.

The continuous use of inorganic fertilizers leads to soil degradation, in which the soil becomes rapidly hardened and less able to store water, thereby reducing crop productivity. Besides, the excessive use of inorganic fertilizers will have an impact on the environment, causing N_2 emissions and water pollution (eutrophication), damaging biota and organisms in soil, and decreasing soil biology.

Soil fertility in modern agriculture can change, which can be caused naturally or as a result of human activity (Price, 2006). Decrease in soil fertility can occur chemically due to nutrient impoverishment such as high transported nutrients that are

not accompanied by nutrient addition to the soil, soil acidification (the decrease in soil pH), loss of organic matter, and increase levels of toxic elements such as Aluminum (Al) and Manganese (Mn) (Hartermink, 2003).

The natural decline in soil fertility is caused by water erosion due to rain, which results in the loss of fertile topsoil and leaves a new or less fertile surface layer. In addition, a decrease in soil fertility can be caused by human actions such as the exploitation of soil nutrients through harvesting all parts of the plant without adequate nutrient supply. Unused crop yields that are not returned to the soil and excessive tillage will also cause accelerated loss of soil organic matter so that the soil is unable to bind nutrients (Hartermink, 2003). Therefore, to increase soil fertility and reduce the use of inorganic fertilizers, an alternative is needed by using organic fertilizers.

Organic fertilizers are fertilizers derived from living things such as plants, animals, or plant residues obtained through decomposition or weathering. Organic fertilizer can be in the form of solid or liquid, which functions as a supply of organic matter to improve soil physical properties including soil structure, soil aggregate, water absorption, soil chemical properties (adding and activating nutrients), and soil biology. The source of organic matter can be compost, green manure, manure, crop residues, and municipal waste (Simanungkalit et al., 2006). One of the organic fertilizers is *C. odorata* compost.

C. odorata is originally from Caribia and America, which is potential enough to be used as a source of organic matter due to its high biomass production. Wardhani (2006) showed that *C. odorata* produced 18.7 tons/ha in fresh form and produced 3.7 kg/ha in dry form. *C. odorata* biomass has so high nutrient content of 2.65% Nitrogen, 0.53% Phosphate, and 1.9% Potassium that it can be used as alternative organic fertilizer (Suntoro et al, 2001). *C. odorata* is an perennial weed that can grow in area of various perennial crops such as cashews, oranges and oil palm. *C. odorata* can grow on infertile areas and have very light seeds enabling it to grow and spread widely.

The success factor in increasing nutrition and nutrient uptake is the provision of *C. odorata* compost. These factors will affect cultivated crops, one of which is lettuce. Compost, in its application, is required in relatively large amounts. However, a high dose of compost will affect the provision of nutrients and nutrient uptake of plants, while the low dose causes the provision of nutrients and nutrient uptake to be meaningless. Therefore, it is necessary to know the right dose of *C. odorata* weed compost. Based on these problems, it is necessary to do research to get the right dose of *C. odorata* weed organic fertilizer to improve nutrition and nutrient uptake in lettuce plants. This research aimed to determine the effect of *C. odorata* weed compost doses on soil and nutrient uptake of lettuce as well as to obtain the best dose of *C. odorata* compost in improving nutrient and their uptake of lettuce plants.

MATERIALS AND METHOD

This research was conducted at the Sustainable Prosperous Rural Farming (P4S) Training Center, Kepuhan, Argorejo, Sedayu, Bantul and Production Management Laboratory, Plant Science Laboratory, Horticulture Laboratory, Ecology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, which was held for three months. The materials used in the study were *C. odorata* leaves, EM-4, rice bran, rice husk, water, sugar cane drops and NPK Phonska (15:15:15).

The research was conducted using a single factor experimental method arranged in a Randomized Complete Block Design (RCBD). The treatments tested were the doses of *C. odorata* weed compost, consisting of 222 grams/pot, 444 grams/pot, and

666 grams/pot, with comparative treatments of 200 ml/pot NPK Phonska (15:15:15), 320 grams cow manure, and without fertilization. The experiment consisted of three blocks with three samples and three units of sample plants within each treatment.

The stages of this research included the making of C. *odorata* weed compost, preparation of planting media, seed preparation and seeding, planting, maintaining, observing, and analyzing data.

Making *Chromolaena odorata* Compost

C. odorata compost was made two weeks before planting with the required material of 85 kg of C. odorata leaves. The making of C. *odorata* compost was done by enumeration so that it is easier to compost, faster to decompose, and easy to flip and breed the bacteria so that the bacteria are active during composting. The bacterial starter was soaked into the media, and the bran was administered and accumulated so that the bacteria were active and hot, accelerating the decay. Next, the reversing aeration was made so that the maturity of the compost was evenly distributed, and the processed air was circulated so that it was not hot and dry. Mature compost was characterized by a decrease in temperature (<40°C), crumb texture, black color resembling soil, and odorless.

Medium Preparation and Planting

Planting media used was silty-clay soil (dominant in Sedayu area) combined with the treatments as previously described as much as 8 kg/pot. The lettuce used was cultivar Green rapids. Planting was done after the seeds were sown for two weeks with two plants per planting hole. Maintenance consisted of watering, subsequent fertilization for NPK Phonska, and weed control. Watering was done intensively every two days unless the soil was in humid conditions. Follow-up fertilization of 200 ml NPK per/pot was done twice, namely in the first week and the second week after planting. The weed control was done manually.

Observations were made in the fourth week after planting. The data were analyzed by using Analysis of Variance at $\alpha = 5\%$ and further tested using Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

RESULTS AND DISCUSSION

Soils are mixture of mineral materials, organic matter, liquids, and gases. Based on the relative proportion of mineral material, organic matter, and pore space, the soil has texture, structure, and chemical properties affecting its potential. Besides, the relatively low investment cost and simple technology in composting also allow the better soil potential (Lim et al., 2019). The results of the soil analysis are presented in Table 1.

The results of soil analysis indicated that the soil used as planting media in this study was silty-clay textured soil with a fraction of clay and silt dominating the soil (Table 1). Soil pH value indicates that the soil was alkaline soil with a pH H2O value that was higher than 6 so that it contained high alkaline saturation, resulting in high available P.

Table 1. Results of analysis of the soil used as planting media

Variables	Value
Clay Texture (%)	40.21
Silt Texsture (%)	31.92
Sand Texture (%)	27.87
Rate of soil (%)	10.81
Permeability (cm/hour)	1.16
pH H_2O	8.10
Organic C (%)	0.80
Cation Exchange Capacity (%)	30.56
Bases saturation (%)	60.21
Volume (gram/cm3)	1.34
Porosity (%)	33.62
Total N (%)	0.18
Available P (ppm)	6.00
Available K (me/100 g)	0.20

Remarks: Data of soil analysis carried out in soil laboratory of Agricultural Technology Assessment Center Yogyakarta

Soil texture is the smoothness or roughness of the soil determined by the type and amount of soil particles (sand, silt, and clay). The results showed that the doses of *C. odorata* compost had a significant effect on the soil texture (Table 2). The application of *C. odorata* compost at a dose of 666 grams/pot resulted in significantly lower clay texture compared to other treatments. The application of *C. odorata* compost at doses of 666 grams/pot and 444 grams/pot produced significantly higher silt fraction compared to the treatment of NPK Phonska, cow manure, *C. odorata* compost at 222 grams/pot, and without fertilization. The application of *C. odorata* compost at a dose of 666 grams/pot and cow manure produced significantly higher sand fraction compared to the treatment of NPK Phonska, *C. odorata* compost at 222 grams/pot and 444 grams/pot, and without fertilization. The application of *C. odorata* at 222 grams/pot and 444 grams/pot showed the same effect on the sand fraction. The NPK treatment resulted in snad fraction that was not significantly different from that of without fertilization.

Table 2. Percentage of soil fractions after treatment

Treatments	Clay (%)	Silt (%)	Sand (%)
C. odorata 222 grams/pot	38.18 b	33.69 b	28.14 bc
C. odorata 444 grams/pot	36.61 c	35.03 a	28.36 b
C. odorata 666 grams/pot	35.69 d	34.92 a	29.39 a
NPK Phonska (15:15:15) 200 ml/pot	39.83 a	32.36 c	27.82 cd
Cow manure 320 grams/pot	38.15 b	32.37 c	29.48 a
Without fertilization	40.00 a	32.43 c	27.70 d
CV (%)	0.86		

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%

Soil permeability is the ability of the soil to pass through the water flow through the pore space. The results indicated that the doses of *C. odorata* compost had a noticeable effect on soil permeability (Table 3.). The application of *C. odorata* compost at a dose of 666 grams/pot and 444 gram/pot produced higher permeability compared to the treatment of NPK Phonska, *C. odorata* compost at 222 grams/pot, and without fertilization.

H_2O is degree of soil acidity related with the sum of weak acid within liquid. The results showed that the weed had a significant effect on the pH of H_2O (Table 3). *C. odorata* compost resulted significantly lower pH H_2O compared to the treatment of NPK Phonska, cow manure, and without fertilization (Table 3). The application of *C. odorata* compost at a dose of 666 grams/pot resulted a lower H_2O pH compared to *C. odorata* compost at a dose of 222 grams/pot and 444 grams/pot because organic matter can decrease pH by 0.21 – 4.57% (Gusain et al, 2018).

Table 3. Permeability, pH H_2O, and organic C after treatment

Treatments	Permeability (cm / hour)	pH H_2O	C-Organic (%)
C. odorata 222 grams/pot	38.18 b	33.69 b	28.14 bc
C. odorata 444 grams/pot	36.61 c	35.03 a	28.36 b
C. odorata 666 grams/pot	35.69 d	34.92 a	29.39 a
NPK Phonska (15:15:15) 200 ml/pot	39.83 a	32.36 c	27.82 cd
Cow manure 320 grams/pot	38.15 b	32.37 c	29.48 a
Without fertilization	40.00 a	32.43 c	27.70 d
CV (%)	0.86		

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%

Organic C content in the soil describes the state of organic matter in the soil. The results indicated that the doses of *C. odorata* compost had a signofocant effect on the organic C in the soils (Table 3.). The application of C. odorata compost at a dose of 666 grams/pot produced significantly higher organic C compared to other treatments (Table 3) because compost can recycle carbon, nitrogen, phosphate, and potassium (Oldfield et al., 2018).

The compost of *C. odorata* can improve soil permeability. The application of *C. odorata* compost at 666 grams/pot and 444 grams/pot produced significantly higher permeability compared to the treatment of NPK Phonska, *C. odorata* compost at a dose of 222 grams/pot and without fertilization. This result is due to the high dose of organic mat-

ter that has the ability to save larger water, thereby moisturizing the soil.

C. odorata compost produced significantly lower pH of H_2O compared to the treatment of NPK Phonska, cow manure, and without fertilization. This result is because organic matters can decrease the pH of soil that is originally alkaline to neutral pH.

C. odorata compost can increase the organic C in the soil. The application of *C. odorata* compost at 666 grams/pot produced significantly higher organic C compared to other treatments. It is because the higher the dose given will result in the higher organic C.

C. odorata compost weed can decrease the texture of the soil clay. Sufficient content of organic matter in the soil can improve soil condition so as not to be too heavy and not too light in soil processing. In wet condition, clay-textured soil becomes sticky, making it difficult to process. The addition of organic matter can simplify the preparation of the soil. The clay-textured soil often experiences crack that is harmful to the development of roots. Thus, the addition of organic matter will reduce cracking.

C. odorata weeds have high nutrient elements content, such as nitrogen, phosphate, and potassium. These elements are essential nutrients for growth that can improve quality of soil chemical properties. The results showed that the dose of *C. odorata* weed compost had a significant effect on soil total nitrogen and nitrogen nutrient uptake of the plants (Table 4).

The application of weed compost can increase the nitrogen content in the soil and nitrogen uptake because composting of garden waste with livestock manure can reduce nitrogen loss and facilitate organic matter humification (Chen et al., 2019). Besides, Wong et al. (2017) added that composting could control the nitrogen loss. *C. odorata* weed compost at a dose of 666 grams/pot produced the highest nutrien content and nutrient uptake. This result is due to the higher doses given that leads to the greater results. Nitrogen uptake in plants was positively correlated with total nitrogen in soil (0.95 **) that supports plant growth. Organic matter increases total nitrogen by 15.61–22.14% (Gusain et al, 2018). The use of compost in agriculture is constrained because of its long-time action and reduced supply of nutrients to the crops. To enhance the content of nutrients available for the plants in the compost, its supplementation with nutrients and inoculation with microorganisms have been proposed (Sanchez et al, 2018).

Table 4. Total N in the soil and nitrogen uptake of lettuce plants

Treatments	Total N in the soil (%)	Nitrogen uptake (%)
C. odorata 222 grams/pot	2.29 d	2.55 e
C. odorata 444 grams/pot	2.83 b	3.04 b
C. odorata 666 grams/pot	3.05 a	3.42 a
NPK Phonska (15:15:15) 200 ml/pot	2.44 c	2.85 c
Cow manure 320 grams/pot	2.32 cd	2.71 d
Without fertilization	0.11 e	2.14 f
CV (%)	3.61	2.63

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%

Table 5. Available P in the soil and P uptake of lettuce plants

Treatments	Available P in the soil (ppm)	P uptake (%)
C. odorata 222 grams/pot	11.67 e	0.18 d
C. odorata 444 grams/pot	20.33 b	0.32 b
C. odorata 666 grams/pot	23.00 a	0.36 a
NPK Phonska (15:15:15) 200 ml/pot	16.33 c	0.26 c
Cow manure 320 grams/pot	14.33 d	0.23 c
Without fertilization	5.33 f	0.16 d
CV (%)	5.52	7.98

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%

The results showed that *C. odorata* weed compost doses gave significant effect on available P in the soil and phosphate uptake of the plant (Table 5). Phosphate uptake in plants was positively correlated with available phosphate in the soil (0.95 **), which supported leaf growth and increased

root growth. Organic matter increases total P by 29.75–50.67% (Gusain et al, 2018).

The results showed that doses of C. *odorata* weed compost significantly affected available soil potassium and potassium uptake of the plant (Table 6). Potassium uptake in plants were positively correlated with the available potassium in the soil (0.95 **), which supported plant growth characterized by plant height, number of leaves, leaf area, shoot fresh weight, shoot dry weight, net assimilation rate, and relative growth rate. Therefore, the absence of potassium affects the assimilate transport (Wijaya, 2008). Organic matter increases total K by 30.3–81.59% (Gusain et al, 2018).

Table 6. Available K in the soil and K nutrient uptake of lettuce plants

Treatments	Available K (me/100 gram)	K uptake (%)
C. odorata 222 grams/pot	0.23 c	1.42 d
C. odorata 444 grams/pot	0.34 a	1.70 b
C. odorata 666 grams/pot	0.36 a	1.93 a
NPK Phonska (15:15:15) 200 ml/pot	0.30 b	1.59 c
Cow manure 320 grams/pot	0.24 c	1.52 c
Without fertilization	0.16 d	1.21 e
CV (%)	4.09	2.43

Remarks: Means followed by the same letters in the same column are not significantly different based on DMRT at α 5%

The application of C. *odorata* weed compost at various deses resulted in higher total nitrogen available P, and available K when compared to NPK Phonska treatment and without fertilization. In addition, C. *odorata* weed compost at a dose of 666 grams/pot produced higher nitrogen uptake, phosphate leaf, and leaf potassium compared to other treatments.

CONCLUSION

The application of C. *odorata* compost significantly increased soil quality and uptake of nitrogen, phosphate, and potassium. C. *odorata* at a dose of 444 grams/pot gave the highest content of nitrogen, phosphate, and potassium in lettuce plants compared to inorganic fertilizer, cow manure, and without fertilizer treatments.

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Growth and Yield Responses of Four Soybean (*Glycine max* (l.) Merrill.) Cultivars to Different Methods of NPK Fertilizer Application

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ABSTRACT

This study aimed to determine the growth and yield responses of four soybean cultivars to different methods of N, P, and K fertilizers application. The methods consisted of one-time application (at the planting time) and split application (at the planting time and during the initial stage of pod formation (R3 stage)). Four superiors soybean cultivars were used, namely Anjasmoro, Grobogan, Dena-1, and Argomulyo. One-time fertilizers application resulted in an increased plant height compared to split fertilizers application. In contrast, the split fertilizers application led to a higher yield of soybeans indicated by the higher total number of pods, number of filled pods, 100-grain weight, and seed dry weight. Cv. Anjasmoro produced higher growth and yield than cv. Grobogan, Dena-1, and Argomulyo. It also produced the highest yield compared to other cultivars. Meanwhile, cv. Argomulyo produced the lowest yield in both fertilization methods. Based on the soil analysis after harvest, the nutrient content of K in the soil was higher than before planting in both fertilization methods. In addition, the split fertilizers application led to higher utilization of P in the soil after harvest.

Keywords: Fertilization; Generative; Soybeans; Vegetative

ABSTRAK

Penelitian ini bertujuan untuk mengetahui tanggapan pertumbuhan dan hasil empat varietas kedelai terhadap cara pemberian kombinasi pupuk N, P, dan K. Cara pemberian kombinasi pupuk N, P, K satu kali saat awal vegetatif dan dua kali yaitu saat awal vegetatif dan fase awal berpolong (R3) diaplikasikan pada empat varietas unggul kedelai yaitu Anjasmoro, Grobogan, Dena-1, dan Argomulyo. Cara pemberian pupuk satu kali menghasilkan pertumbuhan tinggi tanaman yang diukur saat R3 lebih tinggi daripada cara pemberian dua kali sedangkan cara pemberian pupuk dua kali hasil kedelai lebih tinggi daripada cara satu kali. Hasil kedelai ditunjukkan oleh jumlah polong total, jumlah polong isi, bobot 100 butir, dan bobot kering biji. Varietas Anjasmoro menghasilkan pertumbuhan dan hasil lebih tinggi daripada varietas Grobogan, Dena-1, dan Argomulyo. Varietas Anjasmoro menghasilkan hasil tertinggi daripada varietas lainnya (Grobogan, Dena-1, dan Argomulyo) sedangkan varietas Argomulyo menghasilkan hasil kedelai terendah pada kedua cara pemupukan yang diterapkan. Informasi tambahan dari penelitian ini yaitu analisis tanah yang menunjukkan bahwa kandungan unsur hara K di dalam tanah setelah panen ternyata lebih tinggi daripada sebelum tanam. Unsur P di dalam tanah setelah panen terjadi penurunan lebih besar pada cara pemberian pupuk satu kali dibandingkan cara dua kali.

Kata Kunci: Pemupukan; Kedelai; Vegetatif; Generatif

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an agricultural commodity which is widely used, among others as processed food, raw industry material and as refreshment material. It is necessary to increase the soybean production every year as the number of population and industrial developments requiring raw materials of soybean keep increasing. The increase in soybean demand, which has fluctuated in the past five years and tends to increase by 2.49%, is not balanced with the increase in soybean production which only increased by 0.86%, from 954,997 tons/ha in 2014 to 963,183 tons/ha in 2015 (Central Bureau of Statistics, 2015). Therefore, various efforts are required to increase soybean production, one of which is through fertilization.

According to Permanasari (2014), the effective use of fertilizers must fulfil the right five aspects, namely dose, type, time, method of administration, and target. Appropriate dose and proper fertilization method can optimize plant growth and yield thus increasing crop production. The results of the research on soybean responsiveness to N, P, and K fertilizers have so far not been consistent, in aspect of both the doses and the time of administration. N, P, and K fertilizers in soybeans are commonly administered once, which is at planting time. In

this method, soybean production, especially at the level of farmer is still relatively low. The average national soybean productivity is 1.3 tons/ha ranging from 0.6 to 2.0 tons/ha, while at the research level, it reaches 1.7 -3.2 tons/ha, depending on the condition of the land and the technology applied (Edison et al., 2013). Soybeans production is still relatively low despite being given N, P, and K at the recommended doses.

The selection of cultivar, as one of the genetic factors that interacts with the environment, plays an important role in maximizing crop yields. It is stated that internal factors in genetic control vary from one cultivar to another cultivar so that a cultivar suitable for a particular condition is not necessarily suitable for other agro-climate conditions. In addition, each cultivar also has different responses to external factors, such as fertilization. The results of the research conducted by Marliah (2012) reported that cv. Anjasmoro and Grobogan produced higher plant height and seed weight per plant compared to cv. Kipas Merah. Different soybean cultivars gave different responses to the application method of N, P and K fertilizers. Therefore, the aim of this research was to find out the effectiveness of N, P, and K fertilizer application method in improving the growth and yield of soybeans.

MATERIALS AND METHOD

The research was conducted from February 2017 to June 2017 at the Integrated Field Laboratory (Gedong Meneng Campus, Bandar Lampung) and Laboratory of Seed and Plant Breeding, Faculty of Agriculture, University of Lampung. The treatments were arranged in factorial (2x4) randomized complete block design (RCBD) with 3 replications. Grouping was conducted based on planting days, which is 3 days apart.

The first factor was the method of NPK fertilizers application, namely one-time application (P1) at the beginning of vegetative growth and split application (P2) at the vegetative phase and the initial stage of pod formation. The second factor was soybean cultivars consisting of four soybean cultivars, namely Anjasmoro (v1), Grobogan (v2), Dena 1 (v3), and Agromulyo (v4).

The doses of fertilization in this study were 50 kg/ha urea, 100 kg TSP and 100 kg/ha KCl for both application methods. The entire fertilizers in one-time application (p1) were given one week after planting except TSP which was given one week before planting. Meanwhile, the entire doses of fertilzers in split application were divided into two parts. The first half doses were 25 kg/ha urea, 50 kg/ha TSP and 50 kg/ha KCl which were given one week after planting except TSP which was given one week before planting. The other half doses were 25 kg/ha of urea, 50 kg/ha of TSP and 50 kg/ha of KCl which were given in the initial generative phase, which is initial stage of pod formation (R3) or about 7 weeks after planting.

Observations in this study include observing plant growth and production. Variables observed were plant height (cm), plant dry weight (g), total number of pods (pods), number of filled pods (pods), and weight of 100 grains (g). All data were analyzed by Least Significant Difference (LSD) at a 0.05 (5%).

RESULTS AND DISCUSSION

Soybean Production as Affected by Cultivars and Different Methods of NPK Fertilizers Application

There was interaction effect between soybean cultivars and the method of NPK fertilizers application on the number of filled pods and dry weight of seed (t/ha). Both application methods on Anjasmoro resulted in higher number of total pods and dry weight of seeds (ton/ha) (Table 1 and 2).

Affected by one-time application, cv. Anjasmoro produced the highest number of filled pods and dry weight of seed, followed by cv. Grobogan, Dena-1, and Argomulyo, respectively. Likewise, in split application, cv. Anjasmoro also showed the highest number of filled pods and dry weight of seeds, followed by cv Grobogan and Dena-1, and the lowest yield was observed in cv. Argomulyo. However, according to the average effects of the fertilization methods on the four cultivars, it can be seen the split fertilizers application gave a better result.

Table 1. Number of filled pods as affected by soybean cultivars and different methods of NPK fertilizers application

Fertilizers Application	Cultivar			
	Anjasmoro	Grobogan	Dena-1	Argomulyo
One-time (P1)	74,17 a	63,50 b	64.67 b	62.83 b
	B	B	A	A
Split (P2)	89.00 a	74.00 b	70,00 bc	66.50 c
	A	A	A	A

Remarks: Means followed by the same letters, uppercase letters for cultivars and lowercase letters for fertilization methods, are not significantly different based on the 5% LSD Test = 5.74.

Table 2. Dry weight of seeds as affected by soybean cultivars and different methods of NPK fertilizers application

Fertilizers Application	Cultivar			
	Anjasmoro	Grobogan	Dena-1	Argomulyo
One-time (P1)	1.25 a	1.06 b	1.03 b	1.00 b
	B	B	B	A
Split (P2)	2.33 a	1.73 b	1.55 c	1.11 c
	A	A	A	A

Remarks: Means followed by the same letters, uppercase letters for cultivars and lowercase letters for fertilization methods, are not significantly different based on the 5% LSD Test = 0.19.

Growth and Yield Responses of Soybean Cultivars to the Different Methods of NPK Fertilizers Application

One-time fertilizers application produced better plant growth than split fertilizers application as indicated by the plant height. NPK fertilizers application at initial growth phase will affect the availability of assimilates during vegetative growth of the plants. One-time fertilizers application at the initial growth phase was thought to provide more N, P, and K nutrients which were needed in this phase compared to the split fertilization (Table 3). This is in line with Jamili's study (2017) reporting that the application of 50 kg/ha of Urea, 100 kg/ha of TSP, and 100 kg/ha of KCl resulted in a higher growth rate of soybean compared to the application of 25 kg/ha of Urea, 100 kg/ha of TSP, and 100 kg/ha of KCl.

Table 3. Soybean Growth and Yield Response to different methods of NPK fertilizers application

Observation variables	Fertilizers Application		5% LSD
	One-time (P1)	Split (P2)	
Plant height 5 weeks after planting (cm)	35.75 a	33.54 b	2.01
Total number of pods	72.46 b	78.42 a	2.97
100 grain weight	14.64 b	16.84 a	1.58

Remarks: Means followed by the same letters are not significantly different based on the 5% LSD Test.

Nevertheless, the split fertilization obtained highr yields than the one-time fertilization as indicated from higher number of total pods, number of filled pods, 100 grain weight, and dry weight of seed (t/ha) (Table 3). This is presumably because the nutrients needed by plants for yielding are still available due to the second fertilizer application. Nutrients given in the generative phase (initial pod forming) could increase the metabolic process for optimizing the seed production due to the availability of assimilates during maximum seed filling. This result is in line with Tabri's research (2010) reporting that N, P, and K fertilizers application produced the highest weight of 100 grains (g) and the highest seed yield (t/ha) followed by the provision of NK (-P), NP (-K), and PK (-N) fertilizers. This shows that N is needed in the generative phase. The rsults of Saragih's study (2013) revealed that split Urea application at a dose of 100 kg/ha (at a week after planting and early flowering) increased corn yield by 10.65 t/ha. According to Saragih (2013), nitrogen is absorbed by plants during the growing period until the maturation of seeds so that the plant requires continuous N availability in all growth stages until seed formation.

Table 4. Growth and production responses of different soybean cultivars

Observation variables	Cultivars				5% LSD
	Anjasmoro	Grobogan	Dena-1	Argomulyo	
Plant height 5 weeks after planting (cm)	36.52 a	32.27 b	33.03 b	36.11 a	3.01
Stunted dry weight (g)	8.67 a	4.87 c	8.05 ab	7.05 b	1.48
Total number of pods	84.67 a	75,58 ab	72.75 bc	68.75 c	4.20
100 grain weight	17.86 a	15.24 b	15.79 b	14.08 b	2.24

Remarks: Means followed by the same letters are not significantly different based on the 5% LSD Test.

Growth and Yield Responses of Different Soybeans Cultivars.

Cv. Anjasmoro showed higher growth and yield than other cultivars, indicated on higher plant height and dry weight (Table 4). According to Research Institute for Peanuts and Tubers (2017), cv. Anjasmoro can grow up reaching 64-68 cm. Ratnasari's research (2015) reported that cv. Anjasmoro produced the highest plant height at 6 weeks after planting compared to cv. Grobogan. The high growth rate of cv. Anjasmoro in a better production rate as well as on dry weight, total pods, number of filled pods, weight of 100 seeds and dry weight of seeds (t/ha). The difference in characteristics between the four cultivars used can also influence soybean growth and yield. Each cultivar has its own advantages. Zahrah (2011) states that soybean plants have many varieties, each variety will give different responses to growth and production rate. Each variety has genetic characteristics that are not the same, which can be seen from the phenotype and characteristics of each of these varieties. According to Melati et al. (2008), the diversity of soybean seed size in one variety occurs due to the diversity of conditions between plants in the crop, and the diversity of plant conditions so that the weight of soybean seeds is influenced by the size of plant seeds that are influenced by genetic and environmental factors.

Soil Analysis before Planting and after Harvest

The results of soil analysis before planting and after harvest showed that P nutrients in the soil, which initially was 317.18 µg/g, decreased after harvest. The one-time fertilization resulted in P content of 231.15 µg/g, while the split fertilization resulted in P content of 278.28 µg/g. Thus, it was concluded that the soybean plants took available P nutrients in the soil and took part of the P from the added fertilizers.

Table 5. Results of soil analysis before planting

No.	Parameter	Unit	Results
1	P	µg / gdry base	317.18
2	K	µg / gdry base	278.60
3	B	µg / gdry base	36.67
4	Ca	µg / gdry base	2095.07
5	Na	µg / gdry base	131.99
6	Fe	µg / gdry base	56164.25
7	Zn	µg / gdry base	44.86
8	Cu	µg / gdry base	9.90
9	pH	-	6.04
10	PO_4^{-3}	µg / gdry base	636

Table 6. Results of soil analysis after planting and fertilizers application

Sample name / sample code	Test parameters	Unit	Results	Test method
Split fertilized soil / 019 / TNBT / 12 / BL / 12/17	N	% w/w	0.27	AOAC 2001.1
	P	µg/g	278.28	Inhouse Method
	K	µg/g	873.18	Inhouse Method
One-time fertilized soil / 020 / TNBT / 12 / BL / 12/17	N	% w/w	0.22	AOAC 2001.1
	P	µg/g	231.15	Inhouse Method
	K	µg/g	539.57	Inhouse Method

Absorption of K nutrients by soybean plants only takes up little K nutrients that have been added to the soil. The results of soil analysis after harvest showed that there was an increase in K elements in the soil, which increased from 278.60

µg/g to 539.57 µg/g in one-time fertilizers application and 873.18 µg/g in split fertilizers application (Table 5 and 6). this possibility caused the presence of Na in soil could replaces K (Marschner and Cakmak, 1989). Based on these results, it was concluded that the split fertilizers application was efficient because the absorption of the nutrients was less than the one-time fertilizers application.

CONCLUSION

One-time NPK fertilizers application produced better growth than the split fertilization as indicated on plant height. However, the split fertilizers application resulted in higher yields of soybeans by producing higher number of total pods, number of filled pods, weight of 100 grains, and dry weight of seeds. Cv. Anjasmoro produced higher growth and yield than cv. Grobogan, Dena-1, and Argomulyo. Both fertilizers application methods on cv. Anjasmoro produced the highest yield compared to other cultivars. Cv. Anjasmoro and Grobogan produced the highest yields if treated with split fertilizers application, while cv. Dena-1 and Argomulyo did not show different responses to either one-time or two times. Based on the results of soil analysis, the K content after harvest, higher than before planting, while the P content was higher in split fertilizers application.

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Utilization of Diethanolamide Surfactant from Methyl Esters of Palm Oil in Herbicide Formulation with Active Isopropylamine Glyphosate

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ABSTRACT

A surfactant that can be developed from palm oil is non-ionic. Diethanolamine is a nonionic surfactant based on palm oil methyl ester, which can replace the use of polyoxyethylene amine surfactant in a commercial herbicide formula that harms the environment. This research aimed to determine the physicochemical properties of diethanolamine surfactant and to study the effect of diethanolamine surfactant addition in herbicide formulation with active ingredients of isopropylamine glyphosate. This study was arranged in a complete randomized design with surfactant concentrations as treatment, consisting of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 4%, 5%, 6%, 7%, 8%, and 9% (w/w). The results showed that there was a significant effect of diethanolamine surfactant addition on the characteristics of the herbicide formulation. The concentration of 5% diethanolamine surfactant had the best character in producing herbicide with the lowest surface tension and a contact angle of 30.73 dyne/cm and 11.48°. The commercial herbicide, having a surface tension of 36.27 dyne/cm and a larger contact angle of 83.03°, was used as the comparison for the formulations. The stability of the solution was up to 100% for 5 weeks at room temperature with solubility in water of 80.60% and a droplet size of 7.20 µm.

Keywords:Diethanolamine; Isopropylamine glyphosate; Non-ionic surfactant

ABSTRAK

Surfaktan yang dikembangkan adalah surfaktan non-ionik dari minyak sawit. Dietanolamida merupakan surfaktan nonionik dari metil ester kelapa sawit yang dapat menggantikan penggunaan surfaktan polioksitilenamin dalam formula herbisida komersial yang berdampak negatif pada lingkungan. Penelitian ini bertujuan (1) untuk mengetahui sifat fisiko-kimia surfaktan dietanolamida yang dihasilkan, (2) untuk mengetahui pengaruh pemberian surfaktan dietanolamida dalam formulasi herbisida berbahan aktif isopropilamina glifosat. Penelitian ini dirancang menggunakan rancangan acak lengkap satu faktor dengan perlakuan penambahan surfaktan dietanolamida 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% dan 9% (b/b). Hasil penelitian menunjukkan adanya pengaruh nyata dari pemberian surfaktan dietanolamida terhadap karakteristik formula herbisida yang dihasilkan. Perlakuan konsentrasi surfaktan dietanolamida 5% memiliki karakteristik terbaik dalam formula herbisida yang dihasilkan yakni nilai tegangan permukaan dan sudut kontak paling rendah sebesar 30,73 dyne/cm dan 11,48o.. Hasil formula tersebut berbeda nyata dengan herbisida komersial yang memiliki nilai tegangan permukaan sebesar 36,27 dyne/cm dan sudut kontak lebih besar yakni 83,03o. Formula yang dihasilkan memiliki nilai stabilitas penyimpanan pada suhu ruang selama 5 minggu hingga 100% dengan kelarutan formula herbisida dalam air sebesar 80,60%. Ukuran droplet yang dihasilkan sebesar 7,20 µm.

Kata Kunci: Dietanolamida; Isopropilamina glifosat; Surfaktan nonionik

INTRODUCTION

In the herbicide formulation, there is an active ingredient that is added in the form of an adjuvant to get the appropriate formula so that it can increase the effectiveness of the active ingredient used when applied. Herbicide formulations generally have high active ingredients around 25-65%, and the rest are adjuvants (Tominack, 2010). In recent years, many herbicide products have been developed not only to maximize their efficiency and effectiveness in killing weeds but also to minimize the impact of environmental pollution. Ghosh et al. (2014) suggested that one way to improve the performance of active ingredients and reduce the impact of environmental pollution is the addition of surfactants, which have a role in reducing negative impacts on biodiversity conservation.

The use of herbicides through spraying requires a type of surfactant that has wetting properties so that the herbicide solution gets wetter, thus increasing the effectiveness of the herbicide during application without interfering with the stability of the active ingredients used in the herbicide

formula. The role of surfactants can function as active compounds in wetting, dispersing, homogenizing, leveling, and attaching active ingredients to the surface of weed leaves. Surfactants that are often used in herbicide formulas are non-ionic surfactants, and some types of anionic surfactants and types that are not widely used in herbicide formulas are cationic surfactants (Tominack, 2000). The commercial herbicide formula commonly used by farmers usually consists of 48% glyphosate IPA, 15% polyoxyethylenamin surfactant (POEA), and water solvent. Chemical surfactants such as POEA in commercial herbicides exert a highly corrosive effect and have high toxicity to aquatic organisms and animals (Mesnage et al., 2015). Accordingly, it is necessary to find an alternative for safe and environmentally friendly surfactants. For this reason, the Surfactant and Bioenergy Research Center (SBRC) research team of IPB developed a type of non-ionic surfactant namely diethanolamine surfactant synthesized from the amidation reaction between diethanolamine and methyl ester of palm oil fatty acids where the surfactant diethanolamine has the lowest surface tension compared to alkyl polyglycoside, alkylphenol ethoxylates, and lauryl betaine (Suryani et al. 2012).

Recently, the demands for diethanolamine surfactants come from imports based on coconut oil and palm kernel oil, which are estimated to grow continuously due to their wide applications such as in the agrochemical industry, the cleaning industry, and the cosmetics industry. Besides, diethanolamine surfactants are more biodegradable and not toxic to the environment. The development of surfactants from palm oil aims to increase the added value of palm oil derivative products because Indonesia's palm oil production is very high so that the potential for development is very large. Based on the performance of diethanolamine surfactants in pesticides, Nisya et al. (2015) stated that diethanolamine surfactant from methyl ester of palm oil at a concentration of 6% was able to influence the effectiveness of insecticides made from buprofezin. The application of herbicide made from glyphosate IPA is carried out by spraying, which requires a type of surfactant to increase adhesion, especially when applied in the rainy season so that the surface of the weed leaves will get wetter. With the use of surfactants, the leaf surface covered with herbicide becomes wider, and the herbicide lasts longer on the surface of the weed leaf (Tominack, 2000). Herbicide formulation by Singarimbun (2012) using a 10% lauric acid-based diethanolamine surfactant fractionated from PKO and 48% glyphosate IPA produced a formula with a viscous dark yellow visual appearance. The development of diethanolamine surfactants from methyl esters of palm oil is still not widely used in herbicides. For this reason, this study aimed to determine the physicochemical properties of the resulting diethanolamine surfactant and the effect of the addition of diethanolamine surfactant developed from methyl esters of palm oil in herbicide formulations with active ingredients of isopropylamine glyphosate.

MATERIALS AND METHOD

The study was conducted from December 2017 to January 2018 at the Surfactant and Bioenergy Research Center (SBRC) Laboratory of IPB Baranangsiang Bogor, West Java. The materials used in this research were diethanolamine, oil palm olein, 48% glyphosate isopropylamine, distilled water, methanol, KOH, and NaOH 30%. The stages of the research were as follows:

Synthesis of diethanolamine surfactants

Before the synthesis of the diethanolamine surfactant, the raw materials from palm olein were prepared through the transesterification process,

resulting in a product in the form of methyl ester. The methyl ester was then heated to a temperature of 100°C. After that, the diethanolamine surfactant was synthesized through an amidation process by reacting methyl ester and diethanolamine using a 30% NaOH catalyst as much as 1% (w/w) of all the total ingredients. The molar ratio of methyl esters and diethanolamine was 2: 1. The amidation process required ± 4 hours at 140°C with a stirring speed of 300 rpm (Hambali et al., 2014). The results were then analyzed for their physicochemical properties, including density analysis (SOP for Densitymeter Anton Paar DMA 4500M), surface tension (SOP for Spinning drop tensiometer), viscosity (SOP for Brookfield DV-III ultra) and pH (SOP for Schott pH meter).

Herbicide formulation

Herbicide formulation made is a type of soluble liquid formulation with 48% (w/w) glyphosate isopropylamine active ingredients according to commercial herbicide formula and the addition of diethanolamine surfactant in the formula of 1-9% (w/w) (Indrawijaya, 2016). The solvent then was added in the form of water. The weight of the formula for each treatment was 50 grams. The formulation was carried out using a homogenizer at a mixing speed of 2000-3000 rpm for 10-15 minutes (Hambali et al., 2015). The experimental design used in this stage was a completely randomized design with diethanolamine surfactant concentration as treatment, consisting of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% and 9%. The formulation was replicated 2 times. Analysis of the formulas carried out were surface tension (SOP for Spinning drop tensiometer), contact angle (SOP for phoenix 300 Contact angle analyzer), droplet size (SOP for Microscope Leica ICC 50 HD), stability test of the formula for 5 weeks storage (Suryani et al, 2008), and solubility test in the formulation using Gravimetric method (Fardiaz et al., 1992). The successful formula is the formula with solution stability> 80%, otherwise, it is considered unstable because it does not form a perfect solution (Elvina, 2015). Then the results of these observations were compared with commercial herbicides. The formula having the lowest surface tension and contact angle were selected in this study.

All data were analyzed using analysis of variance (ANOVA) at a 95% confidence level and subjected to Duncan's further test at a 5% error level using Statistical Product and Service Solution (SPSS).

RESULTS AND DISCUSSION

Physicochemical Properties of Diethanolamine Surfactants

Diethanolamine surfactant is a type of nonionic surfactant that is synthesized by an amidation reaction. The raw material used can be in the form of fatty acids or methyl esters that are reacted with diethanolamine using a basic catalyst. The surfactant used in this research was the result of palm oil olein synthesis in the form of methyl ester, which was reacted with diethanolamine producing by-products in the form of methanol. Before the herbicide was formulated, an analysis of the physicochemical properties of the diethanolamine surfactant was performed. The analysis aimed to determine the characteristics of the surfactant produced by diethanolamine, therefore determining the effects of the added surfactant on the characteristics of the herbicide formula produced. The characteristics of diethanolamine surfactants tested in this study are presented in Table 1.

Table 1. Physicochemical properties of diethanolamine surfactants

Physicochemical properties	Values
Density	0.9762 g/cm^3
Surface tension	32.21 dyne/cm
Viscosity	257.35 cP
pH	11.10

The diethanolamine surfactant produced showed a yellowish-brown color, and during storage, it experienced compaction. The purity of the DEA surfactant produced cannot be said to be 100%, considering that during storage, there was still very little yellow liquid thought to be the residual methyl ester that had not reacted completely during an amidation reaction. According to Table 1, the diethanolamine surfactant can reduce the surface tension of the water to 32.21 dyne/cm. The surface tension of the diethanolamine surfactant on a laboratory scale ranges from 32.06 to 33.82 dyne/cm (Hambali et al., 2015). Diethanolamine surfactant can reduce surface tension because the surfactant is more likely to dissolve in water, which causes the movement of surfactant molecules to the surface of the water, thereby reducing its surface tension.

The viscosity characteristics are related to density. Density is the weight of a liquid per volume, and viscosity is the resistance to flow in a liquid. Density testing aims to determine the density between molecules in the diethanolamine surfactant produced. If the density has a higher value, then the liquid has a denser viscosity. The diethanolamine surfactants in this study had a density value that was almost the same as commercial coconut oil-based diethanolamine surfactant or called coco diethanolamine, which was equal to 0.995 g/cm^3, and the value was not much different from the results of Meizar's research (2016) of 0.9772-0.9798 g/cm^3. The viscosity of diethanolamine surfactants at room temperature ranges from 232.73 - 267.86 cP (Meizar, 2016). This value is not much different from the viscosity of the diethanolamine surfactants in this study, which was 257.35 cP. The pH value of the diethanolamine surfactants produced was 11.10. These surfactants tended to be alkaline due to the raw materials and catalysts used in the synthesis of diethanolamine surfactants. Diethanolamine had a pH value of 11, and the use of a base catalyst, in the form of NaOH 30%, was as much as 1% of the total material used.

Product of Herbicide Formulation

Surface tension

Surface tension is the pressure from inside that occurs on the surface of a liquid due to the pulling force of molecules down the surface. Liquid surface tension is influenced by several factors, including liquid type, temperature, pressure, and density. Large liquid molecules such as water have a high surface tension due to an increase in intermolecular attractions (Indah, 2018). Measurement of surface tension was following the concentration of treatment, which was 1-9%. According to Figure 1, the surface tension of the herbicide was between 30.73 - 37.68 dyne/cm. There was a significant effect of the diethanolamine surfactant concentration added in the herbicide formula on the surface tension properties. Based on Duncan's further test analysis, the addition of a 5% surfactant in the formula significantly resulted in the smallest surface tension value. It was assumed that the

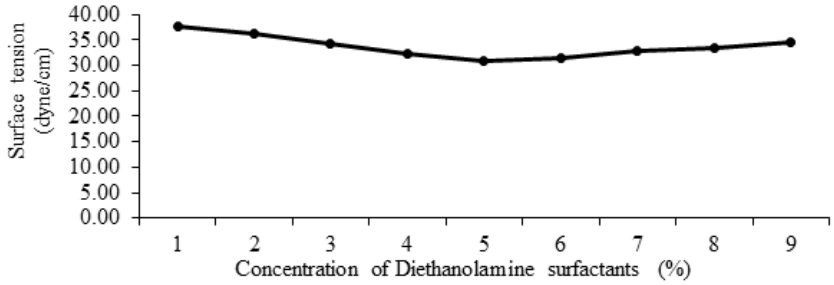

Figure 1. Effects of the concentration of diethanolamine surfactants in the herbicide formula on the surface tension

diethanolamine surfactant was at the optimum condition with the lowest surface tension value at 5-6% because the surfactant concentration had reached the optimum Critical Micelle Concentration (CMC) value (Indrawijaya, 2016). CMC values indicate the critical concentration of surfactants in forming micelles, and the surfactants added in the system will spontaneously turn into micelles. During the formation of micelles in a surfactant solution, the surface tension of the liquid changes rapidly when the concentration of the solution increases, and when it reaches CMC, the surface tension is relatively constant or decreases with the lower slope (Mishra, 2015).

The addition of diethanolamine surfactant at a concentration of 1% - 5% showed a decrease in the surface tension value from 37.68 to 30.73 dyne/cm. Meanwhile, the addition of diethanolamine surfactant at a concentration of more than 5% showed an increase in surface tension, and the highest increase was observed in the concentration of 9%, which was 34.47 dyne/cm. The commercial herbicides showed a surface tension value of 36.27 dyne/cm, which was almost the same with the value in the formulation treatment using a 2% diethanolamine surfactant producing surface tension value of 36.29 dyne/cm. From this comparison, it can be concluded that the addition of a 5% diethanolamine surfactant resulted in the lowest surface tension value compared to commercial herbicides. At the optimum condition, the surface tension value will remain stable even though the surfactant concentration is increased because the concentration has reached a saturation limit, forming a collection of molecules (micelles) in dynamic equilibrium. The surface tension value of herbicide is related to the function of surfactant as an active compound in reducing surface tension. The presence of solvents in the form of water in the formula also influences the value of the surface tension due to the surfactant diethanolamine that is more soluble to water. The presence of a surfactant hydrophilic group bound to molecules on the surface of the liquid leading to this polar solvent causes a reduction in free energy in the solution of the herbicide formula. The reduction in energy occurs due to the division of bond energy between liquid molecules. While the hydrophilic group becomes long, the hydrophobic group of diethanolamine surfactant is shorter, causing the ability of the diethanolamine surfactant in the formation of micelles to decrease. The existence of balanced conditions between molecules on the surface of the liquid and a decrease in the bonding energy between molecules in the liquid by the surfactant causes a decrease in the surface tension value of the herbicide solution. A small surface tension value will increase the ability of a herbicide solution to penetrate into the interior of weed plant tissue. The diethanolamine surfactants that are more likely to dissolve in water then coat the walls of the globules. The role of the diethanolamine surfactant as an active compound to reduce surface tension can be maximum with the smaller and more uniform size of the globule as well as the wider surface of the wall that can be coated by surfactants (Ferdian et al., 2016). The surface tension characteristics of herbicide solutions are closely related to their ability to form contact angles. The smaller the surface tension value, the smaller the attraction between molecules in the solution so that the herbicide solution can spread on the surface of the weed leaves.

Contact angle

The test on the contact angle of the formula aims to show the ability of herbicide solution to quickly spread over the surface of a weed leaf. It was performed to determine the ability of surfactants to stick to and spread the active ingredient of glyphosate isopropylamine on the surface of weed

leaves. This contact angle is formed between the surface of the weed leaf with a dripping herbicide and measured after 10 minutes to see its performance. The average contact angle resulted from the addition of the diethanolamine surfactant in the herbicide formula was between 11.48o to 42.87o. There was a significant effect of diethanolamine surfactant concentration on the contact angles formed. The smallest contact angle was found in the surfactant treatment of 5%, which was 11.48o. Meanwhile, the largest value was observed in the commercial herbicide treatment, which was 83.03o (Figure 2). Commercial herbicides formed a greater contact angle that was almost double that of the formulation treatment using a 1% diethanolamine surfactant, which was equal to 42.87o. The contact angle of commercial herbicides was greater than that of all formulation treatments, presumably due to the use of different surfactants. In commercial herbicides, 15% of POEA surfactants were used. This shows the effectiveness of the diethanolamine surfactant in reducing the contact angle of the formula produced. Conversely, the presence of more POEA surfactants in commercial herbicide formulas shows the ineffectiveness of the POEA surfactant performance on the contact angles formed, as evidenced by the contact angle value of commercial herbicides, nearly reaching 90°C.

According to Figure 2, the addition of DEA surfactants at a concentration of 1% to 5% significantly decreased the contact angle. However, the addition of DEA surfactants at a concentration of more than 5% significantly increased the contact angle. This difference is related to the smallest contact angle value in the addition of a 5% diethanolamine surfactant, indicating that herbicide solution can stick longer and spread faster evenly compared to commercial herbicides that produce a greater contact angle of 83.03o. A small contact angle value is associated with a small surface tension value. The addition of a 5% diethanolamine surfactant resulted in the smallest surface tension value of 30.73 dyne/cm, while the commercial herbicide showed a higher surface tension value of 36.27 dyne/cm. Large concentrations of surfactants will reflect the dispersal behavior of leaves affected by the adsorption process and changes in the interface voltage balance energy (Ivanova and Starov, 2011). The small contact angle shows the surfactant performance as a wetting agent as well as the excellent spread of insecticide liquid on the leaf surface (Zhou, 2007). Contact angle approaching 0o indicates that the drops of herbicide solution can stick and spread very well on the surface of the weed, while the angle approaching 90o indicates that the solution drops on weed leaves can only stick, but the distribution is uneven (Hambali et al., 2015).

Storage stability of the herbicide formula for five weeks

The stability of the herbicide formula solution is one of the important characteristics because it has a significant influence on the quality of the formula produced (Suryani et al., 2000). The herbi-

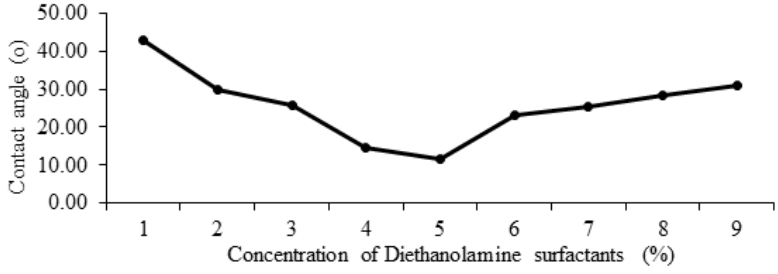

Figure 2. Effects of the concentration of diethanolamine surfactants in the herbicide formula on the contact angle

cide formulation carried out in this study was 48% active glyphosate isopropylamine, diethanolamine surfactant with a concentration of 1 to 9%, and solvent in the form of water. The use of glyphosate isopropylamine active ingredient is following the active ingredient used in commercial herbicide products. Solvents in the form of water were used in this formulation due to the nature of the active ingredient of glyphosate isopropylamine and the nature of the diethanolamine surfactant, which was more soluble to water. Formulas that have been successfully formed are those that have stability of solution> 80%. After formulation, there was no change in the homogeneity. However, after storing for 5 weeks at room temperature, there was a change. Based on the results of ANOVA and Duncan's Multiple Range Test at 5%, there was a significant effect of the concentration of diethanolamine surfactants on the stability of the formula.

The lowest average value of storage stability for 5 weeks at room temperature was observed in the addition of a 1% diethanolamine surfactant, which was 20%. This treatment experienced a separation phase, starting from three days after formulation for 5 weeks. This result is not much different from the addition of diethanolamine surfactants at concentrations of 2% and 3%. Meanwhile, the addition of surfactant at a concentration of 3% showed a stability value of 40.86%, showing perfect stability when compared with the storage stability of commercial herbicides. The addition of a 4% diethanolamine surfactant resulted in stability value that was close to that of commercial herbicide, which was only about 25% difference. This result can be related to the characteristics of diethanolamine surfactants that are dense at room temperature, which can interfere with the stability of the formula. It was seen when the addition of surfactants at concentrations of1% to 4% showed stability during storage of <80% (Table 2). This happens because of the separation phase formed where the water phase and glyphosate isopropylamine active ingredient are at the bottom, and the surfactant phase of diethanolamine is at the top due to the specific gravity of the diethanolamine that is smaller than that of water. On the other hand, the addition of surfactant at concentrations of 5% to 9% showed the stability of up to 100%, as shown by commercial herbicides. This is thought to be due to a balance between the forces that occur, namely the attractive force and repulsion between particles in the formula of herbicide solution. If this equilibrium force is maintained, then the particles in the solution do not unite so that the formula does not become two separate phases. The stability of the solution can reach the maximum if the repulsion force between the globules of the dispersed phase reaches a maximum (Aisha, 2011). If the surface of the newly developed liquid is unstable by surfactant molecules, then hydrophobic interactions can cause the aggregation and instability of the solution (Weiss et al., 2008). During the storage, there were no chemical changes related to the color and odor of the formula produced.

Table 2. Effects of diethanolamine surfactants on the storage stability, droplet size and solubility in water

Surfactants concentration	Average values of herbicide properties	
	Storage stability (%)	Droplet size (μm)
Diethanolamine 1%	20.00 ± 0.00a	9.37 ± 0.07g
Diethanolamine 2%	30.00 ±1.43b	8.31 ± 0.02f
Diethanolamine 3%	42.86 ± 0.00c	8.23 ± 0.03ef
Diethanolamine 4%	87.14 ± 1.43d	7.69 ± 0.34de
Diethanolamine 5%	100 ± 0.00e	7.20 ± 0.02cd
Diethanolamine 6%	100 ± 0.00e	6.93 ± 0.05bc
Diethanolamine 7%	100 ± 0.00e	6.46 ± 0.41b
Diethanolamine 8%	100 ± 0.00e	5.58 ± 0.02a
Diethanolamine 9%	100 ± 0.00e	5.29 ± 0.01a
Commercial herbicide	100 ± 0.00e	5.81 ± 0.08a

Remarks: Means followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at 5%

Droplet size of the herbicide formula

Table 2. explains that the droplet size of the herbicide solution ranges between 5.29 to 9.37 µm. There was a significant effect of the diethanolamine surfactants addition on the droplet size. The addition of a 9% surfactant produced the smallest droplet size of 5.29 µm. The higher the concentration of diethanolamine surfactant used, the smaller the size of the droplet. This is presumably due to the higher intermolecular collision forces as long as the surfactant can still work by covering the droplet surface. Duncan's further test results at 5% explained that the addition of DEA surfactant at concentrations of 8% and 9% showed no significant effect compared to the commercial herbicides. High surfactant concentrations can stabilize the surface of newly developed fluids during the homogenization process, resulting in a smaller particle (Mcclements, 2012).

Meanwhile, the droplet size in commercial herbicide was fairly small, which was 5.81 µm. This value is nearly the same with the values resulted in the addition of diethanolamine surfactants at concentrations of 8% to 9%, ranging from 5.58 µm to 5.29 µm. This difference occurs because the composition of the materials used is different, especially the surfactants. The droplet size affects the performance in the process of penetration and contact with weed surfaces. The smaller the droplet size, the greater the contact surface area with the surface of the weed so that the active ingredients contained in the formula can easily penetrate the weed tissue. Weiss and Muschiolik (2007) stated that an important factor determining the efficiency performance of bioactive materials is particle size because it determines the surface area in its distribution.

Solubility of herbicide in the water

One of the important properties of surfactants is the ability to increase the solubility of ingredients in herbicides, which is related to the application of herbicide in the field. Prior to the application, the herbicide is dissolved in water. Hence, the higher the solubility of the herbicide, the easier the application.

The solubility of the herbicide in this study ranged from 62.55% to 84.55%. Based on the ANOVA results, there was a significant effect of the addition of diethanolamine surfactant on the herbicide's solubility in water. The results of Duncan's multiple range test showed that the higher the concentration of diethanolamine surfactant, the higher the water solubility (Figure 3). The highest percentage of solubility was observed in the addition of diethanolamine surfactant at a concentration of 9%, which was 84.55%. Meanwhile, the commercial herbicides showed a good solubility of 92.35%. On the other hand, the herbicide formulations with the addition of a 1% diethanolamine surfactant showed the lowest solubility of 62.55%. This poor solubility was presumably due to the presence of less homogeneous materials or surfactants in the formulation. The higher the DEA surfactant

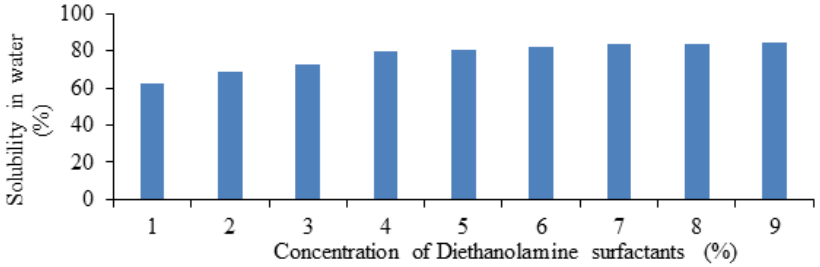

Figure 3. Effects of the concentration of diethanolamine surfactants in the herbicide formula on the solubility in the water

concentration, the higher the solubility. This is likely due to the characteristic of the DEA surfactant that is more polar. These results illustrate that the formulation made in this study nearly reaches the solubility of commercial herbicides. This can be seen from the visual appearance of a commercial herbicide, which is in the form of liquid with golden yellow color and clear.

Good solubility is influenced by the droplet size that is smaller and more homogeneous so that the surface area is increased. Increasing the surface area can increase contact between the herbicide formula with water resulting in higher solubility. The solubility of a compound depends on the size and number of micelles present in the solution (Pilemand, 2002). Generally, there are two types of strengths affecting surfactant molecules in the water, namely, the repulsive force between the hydrophobic parts of a surfactant molecule and the attractive force between water particles of surfactant molecules (Aisyah, 2011). Besides, the solubility in water can also be related to the value of the hydrophilic-lipophilic balance system (HLB) surfactant. A low HLB value indicates the presence of a water-insoluble surfactant, while a high HLB value indicates the presence of a water-soluble surfactant. Diethanolamine surfactant functions as a wetting agent because its HLB value is in the range of 7-9, which is 7.24, and it plays role in reducing the surface tension of the liquid and allowing it to accelerate penetration into the material (Suryani et al, 2000). The higher the concentration of diethanolamine surfactant given, the higher the solubility of the herbicide. This is because diethanolamine surfactants are more likely to dissolve in the water due to their more dominant polar groups so that surfactant molecules will be absorbed more strongly by water than oil, which causes lower surface tension and increased solubility.

CONCLUSION

The best formula made in this research is the addition of a 5% diethanolamine surfactant. The formula has storage stability of up to 100% for 5 weeks, with the solubility in water of 80.60%. The lowest surface tension and contact angle values are lower compared to commercial herbicide treatments, which are 30.73 dyne/cm and 11.48o. The size of the droplet produced is quite small compared to the addition of 1% to 4% diethanolamine surfactant, which is 7.20 µm.

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Genetic Diversity of Potato Based on Random Amplified Polymorphic DNA and Simple Sequence Repeat Marker

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ABSTRACT

Various potato clones are cultivated by farmers in Banjarnegara and Wonosobo, Central Java, Indonesia such as MZ, NH1, NH2, Vega, Gareta, Granola, Bliss, Merah (Red Potato), Ungu (Purple Potato), Klon_17 (K17), Local Dieng, Margahayu, and X. This encourages the importance of genetic diversity and genetic similarities. This study aimed to analyze genetic diversity and genetic similarities between 13 accessions of potatoes based on seven Random Amplified Polymorphic DNA (RAPD) primers and nine pairs of Simple Sequence Repeat Marker (SSR) primers. The results showed that RAPD and SSR primers could be used to analyze genetic diversity and genetic similarities of 13 potatoes accessions from Banjarnegara and Wonosobo. The PLP value was 80.9% in the RAPD primer and 65.41% in the SSR primer. Four RAPD primers were informative based on PIC value: OPG 08, OPM 19, OPP 13, and OPX 04. Three SSR primer were informative: STM 2005, RGH- SSR 8, and StI 035. Genetic similarities presented by Phylogenetic tree analysis resulted in two main clusters. The first cluster consisted of Granola, MZ, Ungu (Purple potato), Merah (Red Potato), Local Dieng, Margahayu, Gareta, Vega, NH2, NH1, Klon_17 (K17), and Bliss. The second cluster consisted of X. Granola and MZ having a high genetic similarity with a genetic distance of 0.07 and 0.132. Meanwhile, K17 and X had a low genetic similarity with a genetic distance of 0.31 and 0.987.

Keywords: Banjarnegara, RAPD, Solanum tuberosum L., SSR, Wonosobo

ABSTRAK

Berbagai klon kentang dibudidayakan oleh petani di Banjarnegara dan Wonosobo, Jawa Tengah, Indonesia seperti MZ, NH1, NH2, Vega, Gareta, Granola, Bliss, Red (Red Potato), Ungu (Ungu Potato), Klon_17 (K17), Lokal Dieng, Margahayu, dan X. Pentingnya keragaman genetik dan kemiripan genetik. Penelitian ini bertujuan untuk menganalisis keragaman genetik dan kesamaan genetik antara 13 aksesi kentang berdasarkan tujuh primer *Random Amplified Polymorphic* DNA (RAPD) dan sembilan pasang *Simple Sequence Repeat Marker* (SSR) primer. Hasil penelitian menunjukkan bahwa primer RAPD dan SSR dapat digunakan untuk keragaman genetik dan kesamaan genetik dari 13 aksesi kentang dari Banjarnegara dan Wonosobo. Nilai PLP adalah 80,9% pada RAPD primer dan 65,41% pada SSR primer. Empat RAPD utama bersifat informatif berdasarkan nilai PIC: OPG 08, OPM 19, OPP 13, dan OPX 04. Tiga SSR primer bersifat informatif: STM 2005, RGH - SSR 8, dan StI 035. Kesamaan genetik yang disajikan oleh analisis pohon Phylogenetic menghasilkan dua kelompok utama. Kelompok pertama terdiri atas Granola, MZ, Ungu kentang, Kentang Merah, Lokal Dieng, Margahayu, Gareta, Vega, NH2, NH1, Klon_17 (K17), dan Bliss. Kelompok kedua terdiri atas X. Granola dan MZ memiliki kesamaan genetik yang tinggi dengan jarak genetik 0,07 dan 0,132. K17 dan X memiliki kesamaan genetik yang rendah dengan jarak genetik 0,31 dan 0,987.

Kata Kunci: Banjarnegara, RAPD, *Solanum tuberosum* L., SSR, Wonosobo

INTRODUCTION

Potatoes are an important horticultural commodity in Indonesia. Nevertheless, the production of potato in Indonesia from 2014 to 2017 continued to decline. According to Central Bureau of Statistics data (2017), potato production in 2014 reached 1,347,728 tons, decreasing to 1,219,277 tons in 2015 and 1,164,738 in 2017. The decline in the potato production also occurred in Central Java, which is one of the potato production centers in Indonesia. Data from the Indonesian Ministry of Agriculture (2017) stated that the number of potato production in Central Java in 2014 reached 292,214 tons, decreasing to 278,552 tons in 2015, 272,976 tons in 2016, and 269,476 tons in 2017. The percentage decreasing in the potato production in Central Java reached minus 1.28%, lower than the percentage decline in potato production in Indonesia which reached minus 9.54% (Ministry of Agriculture, 2017).

In terms of productivity, the number of potato productivity in Indonesia is relatively low. Potato productivity in Indonesia ranges from 15.4 to

18.23 tons/hectare (ha) (Ministry of Agriculture, 2017). Meanwhile, potato productivity in European countries such as Belgium reaches 44.3 tons/ha or the Netherlands reaches 42.2 tons/ha. The value of potato productivity in Indonesia in the range of 2013 to 2016 had increased, but in the range of 2016 to 2017 it came to a decrease. Meanwhile, the value of potato productivity in Central Java in the range of 2013 to 2017 continued to increase (Table 1). The percentage of potato productivity in Central Java upgraded to of 20.17%, in contrast to the percentage of potato productivity in Indonesia which declined up to minus 15.49%. Nevertheless, in general the potato productivity figures in Central Java from 2013 to 2016 were still below the potato productivity in Indonesia nationally.

Table 1. Potato productivity figures in Indonesia and Central Java for the period of 2013-2017

Potato Productivity (ton/ha)	Year				
	2013	2014	2015	2016	2017
Indonesia	16.02	17.67	18.20	18.23	15.40
Central Java	15.51	16.44	17.18	18.25	21.94

(Ministry of Agriculture, 2017)

The decline in potato production and productivity in Indonesia is caused by a number of factors. Some of them are harvested areas which are dropping in numbers and the use of low-quality potato seeds for cultivation. In 2014 the potato harvested area reached 76,291 hectares. However, potato harvested area declined in 2015 (66,983 hectares) and 2016 (66,450 hectares) (Central Bureau of Statistics, 2017). The second factor is due to the low quality of potato seeds used by farmers. Some farmers still use potato seeds from potato cultivation in the previous year. The use of certified superior seeds among potato farmers is still low. One of the reasons is that the existing seed supply system has not run optimally, so it cannot meet the needs of farmers for certified seeds (Fauziyah, 2018). In addition, the price of certified potato seeds is relatively more expensive than potato seeds made by farmers themselves (Sayaka and Hestina, 2011).

To increase potato production and productivity, the development of superior seeds is very important. Besides its long process and stages, developing superior potato also requires basic information such as genetic diversity and similarities among existing potato cultivars. The aim is to find out the superior character of each potato cultivar so that it can be used in potato plant breeding programs.

Analysis of potato genetic diversity and similarities can be carried out with several approaches, such as morphology and molecular. Both approaches make use of a marker owned and become a characteristic of potato. Approach using morphological markers has weaknesses such as long-time requirement, relatively expensive, influence from the environment, and limited diversity (Zulfahmi, 2013). To overcome such disadvantages, the molecular markers come into use.

This technique is based on the use of deoxyribonucleic acid molecules as molecular markers. Molecular markers can directly mark specific genes and can eliminate the influence of environmental factors. Analysis of potato genetic diversity and similarities through the molecular markers approach is divided into several techniques. Two of them are Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) or microsatellite.

RAPD is based on random genomic DNA amplification using the oligonucleotide primer (Kumar et al., 2009). The superiority of the RAPD technique, which does not need DNA sequence information, requires a small quantity of DNA sample (about 5 - 50 ng per reaction); the primer is commercially available, and does not require radioactive (Leksono, 2011; Kumar et al., 2009). SSR is a sequence of 1 to 10 repeat nucleotides tandemly as a composer of the genome repeating

area (Vieira et al., 2016). SSR is most widely used as a molecular marker because it has various advantages, such as specific (Zulfahmi, 2013), highly informative, codominant, high reproducibility, largely abundant, multi-allele and non-radioactive genomes (Kumar et al. , 2009; Miah et al., 2013).

In Banjarnegara and Wonosobo Regency, Central Java, various potato clones have been cultivated by local farmers, some of which are MZ, NH1, NH2, Vega, Gareta, Granola, Bliss, Red Potato, Purple Potato, Klon_17 (K17), Local Dieng, Margahayu, and X. Generally, clones are the result of captive potato farmers in the region and no genetic diversity and similarities are known.

This study aimed to analyse genetic diversity and genetic similarities between 13 accessions of potatoes cultivated in Banjarnegara and Wonosobo district, Central Java Province, Indonesia based on seven RAPD primers and nine SSR primers.

MATERIALS AND METHODS

The materials used were potato leaf samples from Banjarnegara and Wonosobo Regency, Central Java Province, Indonesia (Table 2) and RAPD and SSR oligonucleotides primers (Table 3) (1st base).

Table 2. Potato Accession and the Origin

No	Accession	Origin
1.	MZ	Batur, Banjarnegara
2.	NH1	Batur, Banjarnegara
3.	NH2	Batur, Banjarnegara
4.	Vega	Batur, Banjarnegara
5.	Gareta	Batur, Banjarnegara
6.	Granola	Batur, Banjarnegara
7.	Blis	Batur, Banjarnegara
8.	Merah (Red Potato)	Dieng Kulon, Banjarnegara
9.	Ungu (Purple Potato)	Dieng Kulon, Banjarnegara
10.	Klon 17 (K17)	Dieng Wetan, Wonosobo
11.	Local Dieng	Dieng Wetan, Wonosobo
12.	Margahayu	Dieng Wetan, Wonosobo
13.	X	Dieng Wetan, Wonosobo

DNA Extraction

DNA extraction used a modified CTAB method (Doyle & Doyle, 1987). Potato leaf samples were placed in a mortar and then added with liquid nitrogen and crushed until smooth. Powder samples dissolved with CTAB mixture were then incubated for one hour. The sample suspension was added with 500 µL of chloroform (Merck) mixture: alcohol isoamyl (24:1) and then precipitated with 32 µL of ammonium acetate (Merck) and 233.28 µL of isopropanol (Merck) and incubated overnight at 60oC. The pellets were added with 500 µL of 70% ethanol (Merck), 500 µL of 96% cold ethanol (Merck) and 200 µL of ammonium acetate (Merck). After drying, the sample was added with 2 µL of RNAse (2.5 µg/mL)(Geneaid) and 50 µL of nuclease-free water (Promega). The samples were then ready for next analysis.

DNA Amplification by Polymerase Chain Reaction

The RAPD reactions were performed in a final volume of 25 µL. The mixture contained 1 µL of DNA (50 ng/µL), 3 µL of RAPD primer (10µM) (1st base), 5 µL of 5x My Taq Red Reaction Buffer (Bioline), 0.5 µL of My Taq HS Red DNA Polymerase (Bioline), 15,5 µL of Nuclease-Free Water (Promega). This method refers to Yaycili and Alikamanoglu (2012). PCR program for RAPD primers was presented in Table 4. PCR products were electrophoresed on 1% of agarose gel, about 45 minutes at 75 volts.

The SSR reactions were carried out with 25 µL final volume. The mixture contained 1 µL of DNA (50 ng/µL), 0.75 µL of forward and reverse primer (10 µM)(1st base), 5 µL of 5x My Taq Red Reaction Buffer (Bioline), 0.5 µL of My Taq HS Red DNA Polymerase (Bioline), and 17 µL of Nuclease-Free Water (Promega). The PCR program for SSR primers is presented in Table 5. PCR products were electrophoresed on 3% of agarose gel about 55 minutes at 100 volts.

Data Analysis

PCR results were converted to binary data. Number 1 was if the DNA band appeared and 0 was if no DNA band appeared. The data were used to determine the value of Polymorphic percentage (PLP) and Polymorphic Information Content (PIC), and to reconstruct phylogenetic trees represented in genetic similitaries. The formula to know the PLP and PIC was as follow:

$$PLP = \frac{\Sigma(LP)}{\Sigma(LP)+\Sigma(LM)}$$

Note :

Σ(LP) : sum of polymorphic locus

Σ(LM) : sum ofmonomorphic locus

$$PIC = 1-\Sigma Pij^2$$

Note:

Pi : frequency of j pattern were obtained by primer i

Phylogenetic trees reconstruction based on *Unweighted Pair Group Method with Arithmetic* (UPGMA) and *Maximum Composite Likelihood* used software Mega in 6.0 version (Tamura et al., 2013).

RESULTS AND DISCUSSION

RAPD Analysis

The DNA isolated from the potato leaf sample was amplified with the RAPD primer to determine its profile so that it could be used for genetic diver-

Table 3. RAPD and SSR oligonucleotide primers

No	Name	Sequence	References
1	OPG 08	TCACGTCCAC	Rocha et al., 2010
2	OPG 13	CTC TCC GCC A	Rocha et al., 2010
3	OPJ 13	CCA CAC TAC C	Rocha et al., 2010
4	OPM 19	GTCCGTACTG	Rocha et al., 2010
5	OPN 02	ACCAGGGGCA	Rocha et al., 2010
6	OPP 13	GGAGTGCCTC	Rocha et al., 2010
7	OPX 04	CCG CTA CCG A	Rocha et al., 2010
8	STM 1052	F : CAATTTCGTTTTTTCATGTGACAC R : ATGGCGTAATTTGATTTAATACGT	Ghislain et al.,2004
9	STM 2005	F : TTTAAGTTCTCAGTTCTGCAG R : GTCATAACCTTTACCATTGCTGGG	Milbourne et al., 1997
10	STM 3012	F : AAT TCT ATC CTC ATC TCTA R :CAA CTC AAA CCA GAA GGC AAA	Ghislain et al.,2004
11	STM 3015	F : AGC AAT AAA GTC AAC ACT CCA TCA R :AAT GAA TTA GGG GGA GGT GTG	Ghislain et al. 2004
12	RGH-SSR 8	F : GAATTTTCTCCACTGGCAGC R : TCCAAGGAAACAAAACTTGACC	Bakker et al., 2011
13	RGH-SSR 48	F : AAT TCT TTGAAA TTG GCC CC R : CAC ACC CAACAATCT TTCCC	Bakker et al., 2011
14	POT 083	F : GGGACATCACAGTCT R : GGTGCTCCTATTGGTG	Salimi et al., 2016
15	StI053	F : TCAGACCGGGTTCGATGG R : CGGCTTGAATCATTGCCCA	Feingold et al., 2005
16	StI055	F : CCGTTGATGGGATTGCACA R : TGATATTAACCATGGCAGCAGC	Feingold et al., 2005

Note: 1-7: RAPD primer, 8-16: SSR primer; F: forward primer, R: reverse primer

Table 4. PCR program for RAPD

Step	Temperature (°C)	Time (second)	Cycle (times)
Pre-Denaturation	95	60	1
Denaturation	94	10	34
Annealing	36	30	34
Extension	72	30	34
Final Extension	72	420	1

(Williams et al., 1990; Rocha et al., 2010)

Table 5. PCR program for SSR

Step	Temperature (°C)	Time (second)	Cycle (times)
Pre-Denaturation	95	180	1
Denaturation	95	60	30
Annealing	57	30	30
Extension	72	60	30
Final Extension	72	600	1

(Salimi et al.,2016)

sity and similarities analysis. Examples of RAPD analysis with primer OPG 08 primers are presented in Figure 1.

Of the seven RAPD primers used, all primers showed polymorphisms with the appearance of DNA bands with different sizes from a range of 250 bp to 1600 bp. The example was the results of RAPD analysis with primer OPG 08 (Figure 1). There were five DNA bands for NH2, Local Dieng and Ungu samples with a size of about 250 bp, 400 bp, 550 bp, 750 bp, and 1000 bp, four DNA bands for Red sample with a size of about 375 bp, 550 bp, 750 bp, and 1000 bp, four DNA bands for NH1, Margahayu, and MZ sample with a size of about 400 bp, 550 bp, 750 bp, and 1000 bp, three DNA bands for Gareta and X sample with a size of about 400 bp, 750 bp, and 1000 bp, one DNA band for Vega sample with a size of about 400 bp, three DNA bands for Granola sample with a size of about 250 bp, 400 bp, and 550 bp, two DNA bands for K17 sample with a size of about 375 bp, 550 bp, and 1000 bp, and bands for Bliss sample with a size of about 375 bp and 550 bp.

The results of polymorphism using RAPD primers were produced in accordance with the results of analysis of several similar primers conducted by Rocha et al. (2010) on 16 commercial potato cultivars in Brazil.

SSR Analysis

Isolated DNA from potato leaf samples were also amplified with SSR primers for analysis of genetic diversity and similarities. Examples of the results of SSR analysis with primer STM 1052 was presented in Figure 2. From the nine pairs of SSR primers used, all primers showed polymorphisms with the appearance of DNA bands with different sizes from a range of 150 bp to 200 bp. The example was the results of the SSR analysis by primer STM 1052 (Figure 2a); there was one DNA band appearing at a length of about 200 bp for NH2 and Vega samples, two DNA bands at a length of about 150 bp and 200 bp for Merah, NH1, X, Margahayu, Local Dieng, Granola, Ungu, MZ, and K17 samples,

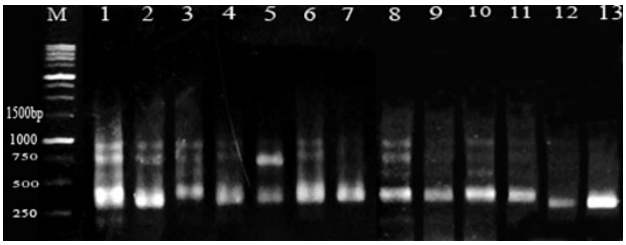

Figure 1. Results of RAPD analysis of 13 potato accessions using primer OPG 08. M: 1 kb DNA Ladder, 1: NH2, 2: Merah, 3: NH1, 4: Gareta, 5: X, 6: Margahayu, 7: Vega, 8: Local Dieng, 9: Granola, 10: Ungu, 11: MZ, 12: K17, 13: Bliss. There are polymorphisms in the sample analyzed

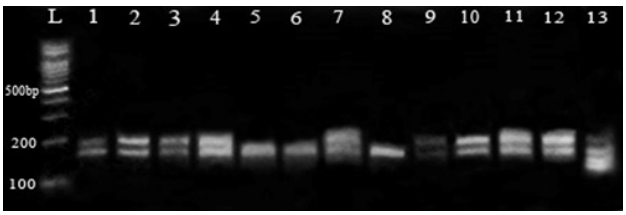

Figure 2. Results of SSR analysis with primer STM 1052. M: 100 bp DNA Ladder, 1: NH2, 2: Merah, 3: NH1, 4: Gareta, 5: X, 6: Margahayu, 7: Vega, 8: Local Dieng, 9: Granola, 10: Ungu, 11: MZ, 12: K17, 13: Bliss. There were polymorphisms in the sample analyzed

and two DNA bands appeared at a length of about 175 bp and 200 bp for Gareta samples.

Genetic Diversity Based on PLP and PIC Value

Based on Table 6, genetic diversity analysis in 13 accessions of potatoes using 7 RAPD primers could detect 67 alleles with a mean of 9.57 alleles per primer. The mean of alleles detected in this study was lower than that of Collares et al. (2004) which yielded 10.75 alleles per primer on detection in 29 accessions of potatoes using 4 RAPD primers. However, it was higher when compared with the results of Hoque et al., (2013) which yielded 4.87 alleles per primer. A total of 55 alleles were polymorphic or 80.9% of the total allele. These results did not differ significantly from the results of Onamu et al. (2016) which characterized 35 accessions of potatoes with 19 RAPD primers. However, this result was still lower than Yasmin et al. (2006) study which analyzed 6 potato cultivars using 3 RAPD primers with PLP value of 94.29%. The highest PLP value was obtained by OPG 08 and OPM 19, which was 100%, while the lowest was by OPN 02 in 42.9%. PLP information was essential to determine the level of genetic variation in a population. The higher the PLP value, the higher the level of variation would be (Kawengian et al., 2016). Polymorphic bands were obtained from differences in PCR band size. Each primer produced a different polymorphic pattern, because each primer produced the DNA band at different base sizes (Sinaga et al., 2017).

For PIC values, seven RAPD primers yielded values ranging from 0.31 to 0.78 with an average of 0.54. The PIC values were lower when compared with the results of Rocha et al., (2010) resulting in a mean of PIC values of 0.9 with the same primer in the analysis of 16 potato cultivars. This difference is thought to be due to the low repetition of RAPD analysis (Jones et al., 1997), thus allowing for variations in results.

According to Nugroho et al., (2015), PIC values are values that inform the level of polymorphism of a molecular marker. PIC also illustrates the level of efficiency of a marker to distinguish genotypes. The PIC value of > 0.5 indicated informative marker which was useful for distinguishing genotypes, while the PIC value of <0.5 was less informative or less efficient in distinguishing genotypes. Based on these criteria, 4 RAPD primers were classified as informative, i.e., OPG 08, OPM 19, OPP 13, and OPX 04, while the other 3 primers were classified as less informative, i.e., OPG 13, OPJ 13 and OPN 02.

According to Table 7, nine SSR primers succeeded in amplifying 28 alleles with a mean of 3.11 alleles per primer. 19 alleles showed a polymorphic band pattern with a mean of 65.41%. StI053 and StI055 showed the highest PLP value, which

Table 6. PLP dan PIC of RAPD

Primer	Sum of locus (alel)	Polymorphic locus	PLP (%)	PIC
OPG 08	6	6	100	0,60
OPG 13	11	7	63,6	0,31
OPJ 13	7	6	85,7	0,46
OPM 19	12	12	100	0,78
OPN 02	7	3	42,9	0,42
OPP 13	13	12	92,3	0,68
OPX 04	11	9	81,8	0,54
Total	67	55		
Average (mean)	9,57	7,85	80,90	0,54

Table 7. PLP dan PIC of SSR

Primer	Sum of locus (alel)	Polymorphic locus	PLP (%)	PIC
OPG 08	3	2	67,0	0,49
OPG 13	3	2	67,0	0,77
OPJ 13	2	1	50,0	0,26
OPM 19	2	1	50,0	0,47
OPN 02	7	5	71,4	0,68
OPP 13	3	1	33,3	0,09
OPX 04	2	1	50,0	0,47
StI053	3	3	100,	0,69
StI055	3	3	100,	0,32
Total	28	19		
Average (mean)	3,11	2,11	65,41	0,47

was 100%, while RGH-SSR 48 was the lowest at 33.3%. This result was lower than Favoretto et al., (2011) which yielded 46 polymorphic alleles with a mean of 4.6 per primer using 10 SSR primers. This result was also lower than Hubert et al. (2015) that reported 42 detectable alleles and 37 alleles or 88% polymorphic on identification of 11 Indian potatoes using 10 SSR primers.

Table 6 also showed the PIC values generated from SSR primers ranging from 0.26 to 0.77 with a mean of 0.47. The lowest value was STM 3012 and the highest one was STM 2005. This result was different from the result of Nugroho et al. (2015) which resulted in PIC values ranging from 0.41 to 0.76 with a mean of 0.59 in the analysis of 14 accessions of potatoes using 14 SSR primers. However, these results were no different from those of Salimi et al. (2016) which resulted in a mean of PIC of 0.42. Of the 9 SSR primers, 3 primers were informative because they showed the PIC value of > 0.5 in STM 2005, RGH-SSR 8, and StI035. Meanwhile, 6 primer PIC values were less informative because the values were < 0.5 as in STM 1052, STM 3012, STM 3015, RGH-SSR 48, POT 083, and StI055.

Genetic similarities

According to Figure 3, analysis of genetic similarities using RAPD primers in 13 potato accessions based on the UPGMA method found that the closest genetic distance was found in the Granola and MZ accessions, which was 0.07. Based on the Maximum Composite Likelihood method, the closest genetic distance for Granola and MZ was also produced with a genetic distance of 0.132 (Figure 4). This indicates that these two accessions had a high genetic similarity. It is known that Granola and MZ were produced from the same elders, Granola (Ministry of Agriculture, 2014). These molecular data were supported by morphological data that there was a similarity between the

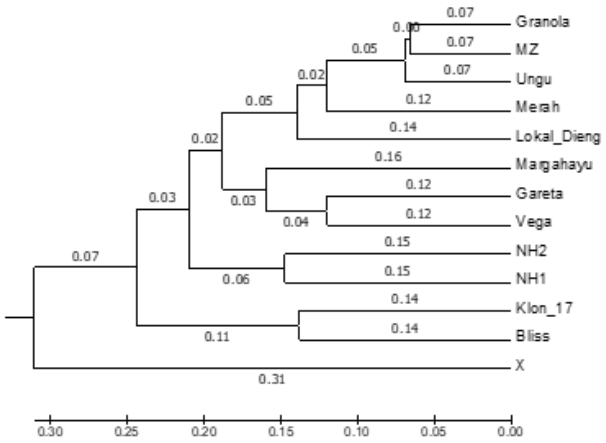

Figure 3. Dendrograms representing the genetic similarities obtained based on RAPD and SSR markers in 13 potato cultivars generated by UPGMA

morphology of Granola and MZ such as large oval leaves, feathers on the leaves, purple flowers, yellow bulb skins, and yellowish fleshy bulbs.

Another interesting thing is in the Ungu accession (purple potatoes). This potato is the result of breeding performed by local potato seed breeders from various existing superior varieties. Based on information from local farmers, one of the elders of purple potatoes was the MZ variety. The results of genetic diversity analysis using the RAPD primer supported this information. Purple potatoes and MZ had high genetic similarities. From the dendrogram presented in Figure 3, the genetic distance data of purple potato and MZ was 0.07, equal to the genetic distance between Granola and MZ. Although morphologically the color of tuber between them was different. MZ has bright yellow tuber , while Ungu has purplish tuber.

Referring to Figure 3, it is also produced that the farthest genetic distance was found in potato accessions of K17 and X. Both genetic distances were 0.31. The data were also supported by analysis based on the Maximum Composite Likelihood method which also produced the farthest genetic distance for K17 and X, with genetic distance of 0.987 (Figure 4). This indicates that both accessions shared low genetic similarities. Based on personal discussions with potato seed breeders in

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. NH2													
2. NH1	0.295												
3. Merah	0.357	0.295											
4. Gareta	0.403	0.482	0.315										
5. X	0.651	0.612	0.576	0.403									
6. Margahayu	0.790	0.403	0.295	0.380	0.428								
7. Vega	0.454	0.336	0.315	0.240	0.651	0.258							
8. Lokal_Dieng	0.336	0.403	0.336	0.482	0.482	0.357	0.482						
9. Granola	0.454	0.380	0.240	0.403	0.576	0.380	0.315	0.258					
10. Ungu	0.428	0.454	0.223	0.380	0.543	0.454	0.380	0.207	0.132				
11. MZ	0.380	0.357	0.258	0.428	0.693	0.357	0.295	0.315	0.132	0.146			
12. Klon_17	0.357	0.482	0.454	0.576	0.987	0.543	0.512	0.380	0.357	0.380	0.223		
13. Bliss	0.403	0.482	0.651	0.576	0.848	0.693	0.512	0.790	0.454	0.543	0.380	0.276	

Figure 4. Genetic distance using the Maximum Composite Likelihood method. The closest distance was 0.132 between accession of Granola and MZ, while the farthest distance was 0.987 between accession K17 and X

the research location, accession X is not yet known as which parent. The morphological character of accession X was also different compared to other accessions cultivated in Batur Banjarnegara and Dieng Wonosobo.

Based on the results of the phylogenetic tree by RAPD dan SSR analysis presented in Figure 3, potato accession from Banjarnegara and Wonosobo were classified into two main clusters. The first cluster consisted of Granol, MZ, Ungu (Purple potato), Merah (Red potato), Local Dieng, Margahayu, Gareta, Vega, NH2, NH1, Klon_17 (K17), and Bliss. The second cluster consisted of X. Potato accessions in the same cluster which had greater genetic similarity than different clusters.

CONCLUSIONS

The results showed that RAPD and SSR primers could be used to analyze genetic diversity and genetic similarities in 13 potatoes accessions from Banjarnegara and Wonosobo. The PLP value was 80.9% for RAPD and 65.41% for SSR. Four RAPD primers were informative: OPG 08, OPM 19, OPP 13, and OPX 04. Three SSR primers were also informative: STM 2005, RGH- SSR 8, and STI 035. Phylogenetic tree analysis yielded two main clusters. The first cluster consisted of Bliss, Grannola, Vega, MZ, Margahayu, Gareta, NH1, NH2, Purple Potato, Red Potato, and Local Dieng. The second cluster consisted of X. Granola and MZ which had a high genetic similarity with a genetic distance of 0.07 and 0.132 respectively, while K17 and X had a low genetic similarity with a genetic distance of 0.31 and 0.987 respectively.

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