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Fakultas Pertanian Universitas Padjadjaran
Gedung Budidaya Pertanian Lt. 3
Jl. Ir. Soekarno Km. 21, Jatinangor, Sumedang 45363
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Website: jurnal.unpad.ac.id/kultivasi

Email: jurnal.kultivasi@unpad.ac.id

PREFACE

Kultivasi volume 22 number 2 is the second issue in 2023, which contains full articles in English. The number of published articles is 14 research articles and reviews originating from various scientific sectors in the agricultural area, such as plant production, soil science, plant breeding, and plant protection.

This edition also started containing articles from international authors to provide new information on agriculture worldwide. This knowledge diversity will add to the richness of the repertoire in agriculture and further advance the research and publications of researchers and academics.

Kultivasi Team strives to improve the quality of articles for heading to an international reputable journal level. We hope to continue providing the best service for fellow authors, reviewers, editors, and stakeholders in the agricultural sector. We tremendously appreciate researchers, students, and academics who always support us by submitting the manuscript to our journal.

August 2023

Editorial Team

AUTHOR'S INSTRUCTIONS

Manuscript that met scientific requirements can be published. The original manuscript is sent to the editor in accordance with the writing requirements as listed below. Editors have the right to change and suggest improvements in accordance with the norms of science and scientific communication. Editors cannot accept papers that have been published in other publications.

The manuscript is typed on Microsoft Word software, on A4 size paper with a writing length ranging from 6-15 pages and followed the template. The manuscript in the Jurnal Kultivasi can be written in English with an effective and academic language style.

The full manuscript is sent to the editors accompanied by a cover letter from the author. The sent manuscript is a group of original paper, soft file of images and other supplementary materials. The editor issues the letter of manuscript acceptance to author once the paper is considered to be going to publish.

Special Requirements

Review Articles:

Articles should discuss critically and comprehensively the development of a topic that is actual public concern based on new findings supported by sufficient and up-to-date literature. Before writing an article, it is recommended that the author contact the Chairman of the Editorial Board for clarification of the selected topic.

The systematics of writing peer articles consists of: Title, author's name and correspondence address; Abstract with keywords; The Introduction contains justifications for the importance of the topic being discussed; Subject matter; Conclusion; Acknowledgment; and References.

Research Articles:

The original manuscript is compiled on the basis of the following sections:

Title

- The title must be brief and indicate the identity of the subject, the purpose of the study and contain keywords and be written in Bahasa Indonesia and English. Titles range from 6-20 words, created with capital letters except for latin names written in italics.

Author's name

The authors must list the name without the title, profession, agency and address of the place of work and the author's email clearly in accordance with applicable ethics. If it is written by more than one author, the writing of the order of names should be adjusted according to the contribution level of each author. The writing of the name of the first author is written the last syllable first (although not the surname), while the subsequent author the initial syllable is abbreviated and the next syllable is written in full. For example: Tati Nurmala and Yudithia Maxiselly then written as Nurmala, T. and Y. Maxiselly

Abstract

- Abstract is an informative writing that is a brief description about the background, objectives, methods, results and conclusions. Abstract is written in English with a maximum of 250 words and equipped with keywords.

Introduction

- Introduction presents the background on the importance of research, underlying hypotheses, general approaches and research objectives as well as related literature reviews.

Materials and Method

- Materials and Methods contains an explanation of the time, place, technique, design, plant material and other materials of experiment as well as statistical data analysis. It should be written in detail so that it is repeatable and reproduceable. If the method used is known in advance then the reference should be listed.

Results and Discussions

- Results and discussions are briefly outlined assisted by informative tables, graphs and photographs. The discussion is a brief and clear review of research results and refers to previous related literatures. Table or Figure Captions are written in English.

Conclusion

- Conclusion is the final decision of the conducted research and the follow-up advice for further studies.

Acknowledgment

- Acknowledgment to sponsors or parties who support the research briefly.

Reference

There are at least 20 references from the last 10 years. The references list all related libraries along with the aim of making it easier to search for readers who need it. Only list libraries that have been published either in the form of textbooks or scientific articles. Using an internationally applicable article author's name writing system. Inside the text, the reference should be written as follows:

- Two authors: Tati Nurmala and Yudithia Maxiselly *then written* Nurmala and Maxiselly (2014) or (Nurmala and Maxiselly, 2014).
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- Book writing format: Author's Last Name. Initials of the year it was published. Book title (each initial letter of a word is written in capital letters, except for conjunctions/prepositions; Edition if the edition is more than one). Name Publisher. Place published.

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Example: Zakry FAA, Shamsuddin ZH, Rahim KA, Zakaria ZZ, Rahim AA. 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ¹⁵N isotope dilution technique. *Microbes Environ.*, 27: 257-262.

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Wicaksono FY · Khairunnisa S · Irwan AW · Nurmala T

The addition of phosphorus and potassium fertilizer in the generative stage of Job's tears affects yield components, yield, and yield quality

Abstract. Job's tears (*Coix lacryma-jobi* L.) is an indeterminate food crop that requires more than one-time application of fertilizer. This study aims to determine the effect of additional doses of phosphorus and potassium at the beginning of the generative phase as side dressing fertilization on yield components, yield, and yield quality of the Job's tears plant. The research was conducted in dry season March - August 2021 at the Experimental Field of the Faculty of Agriculture, Universitas Padjadjaran at Jatinangor, Sumedang Regency around 750 m above sea level. The experimental design used randomized block design (RBD) consisting of nine treatments and three replications, namely P and K fertilizers, respectively at doses of: 0, 20, 30, 40, and 50 kg/ha through one or two frequencies of fertilization. Data analysis used analysis of variance and Scott-Knott test at 5% significance level. The results showed that the application of phosphorus and potassium fertilizers affected the number of panicles, seed weight, and harvest index, but no one effect on other yield components and yield quality. The yield component and the Job's tears yield were decreased compared to previous studies, which were carried out in sufficient water conditions.

Keywords: Drought · Job's tears · Phosphorus · Potassium

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Wicaksono FY¹ · Khairunnisa S² · Irwan AW¹ · Nurmala T^{1*}

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang km. 21, Sumedang 45363, Indonesia

² Study Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang km. 21, Sumedang 45363, Indonesia

*Correspondence: tati.nurmala@unpad.ac.id

Introduction

Indonesia's population growth is increasing from year to year. Based on Indonesia Statistics (2021), Indonesia's population in September 2020 was 270.2 million people. The average population growth rate of Indonesia increased by 1.25 percent per year in 2010-2020. Certainly, increasing population will increase food needs (Prosekov & Ivanova, 2018). So far, most Indonesians consume rice as a staple food (Widyanti et al., 2014). Thus, developing local food through the food diversification program is the government's effort to strengthen community food security (Rozaki, 2020).

Job's tears (*Coix lacryma-jobi* L.) is an alternative food crop that has good nutrition, health benefit, and easy to cultivate in tropical area, especially Indonesia (Nurmala et al., 2017). Job's tears seeds contain 76.40% carbohydrates, 7.90% fat, and 14.10% protein (Grubben et al., 1996). In addition, Job's tears can also be used as a functional food to treat diseases such as diabetes, kidney and liver diseases, lung cancer, and rheumatism (Irawanto et al., 2017). Origin of Job's tears is from Southeast Asia so that the climate in Indonesia is suitable for the growth of Job's tears (Iamsupasit, 1996).

The yield and harvest index of Job's tears from farmers production are still low (Wicaksono et al., 2022). This problem caused by failure of fertilizer dosage and fertilization method, farmers usually give one time fertilization. Job's tears is indeterminate plant that requires more than one time fertilization like other indeterminate plants (Kaschuk et al., 2016). Thus, the Job's tears plant requires additional fertilization in its growth phase, especially the generative stage which determines yield of seed.

Generative stage of plants need phosphorus and potassium fertilizer (Szczepanek & Siwik-Ziomek, 2019). Phosphorus is needed for the formation of ATP, germination, photosynthesis, respiration, growth of young plants, growth of roots and leaves, flowering and seed formation, storing dry matter in cereal seeds, and increasing yield components, yield and yield quality (Gahoonia et al., 1999; Chaichi et al., 2008; Malhotra et al., 2018; Seneweera & Conroy, 1997), while potassium is needed to fight pests and diseases, photosynthesis, osmoregulation, enzyme activation, protein

synthesis and ion transport (Dhillon et al., 2019; Zörb et al., 2014). Potassium also affects the yield, yield, and quality of seeds (Brennan & Bolland, 2009; Ali et al., 2003).

The application of additional fertilizer in generative stage of Job's tears has never been studied. It is expected that yield and quality of Job's tears seeds will increase with the addition of this fertilizer.

Materials and Methods

The research was carried out from March to August 2021 at the Experimental Field of the Faculty of Agriculture, Universitas Padjadjaran in Jatinangor, Sumedang. The altitude of the research location is around 750 m above sea level. The research site has a temperature of 21.8 – 23.0°C, humidity 86 – 91%, and belongs to the C3 agro-climatological zone according to Oldeman's classification. Rainfall during the study was 490 (March), 44.5 (April), 120.5 (May), 85.5 (June), 16 (July), and 10 mm (August). The site soil had pH 7.22 (neutral), organic C 1.26% (low), total N 0.11% (low), C/N 11.45 (high), total P₂O₅ 34.98 mg/100 g (moderate), available P₂O₅ 1.95 ppm (very low), K₂O 4.44 mg/100 g (very low), and cation exchange capacity 24.96 (moderate).

The materials that used in this research were Job's tears seeds cv. Watani Wado, chicken manure, NPK fertilizer (15:15:15), SP-36 fertilizer (36% P), KCl fertilizer (55% K), profenofos insecticide, and mancozeb fungicide. The tools used in this study were tape measure, analytical balance, caliper, Wagner penetrometer, farming equipment, and AGR-RM40 dehuller machine.

The research was an experiment, which used a randomized block design (RBD) with nine treatments and three replications, so there were 27 plots. Each plot was 12 m² that consisted of 50 plants. The treatment was:

A = without additional P and K (control),

B = additional P and K 20 kg/ha, respectively, in one time application at 16 weeks after sowing (WAS),

C = additional P and K 20 kg/ha, respectively, in two times application at 16 and 18 WAS,

D = additional P and K 30 kg/ha, respectively, in one time application at 16 WAS,

E = additional P and K 30 kg/ha, respectively, in two times application at 16 and 18 WAS,

F = additional P and K 40 kg/ha, respectively, in one time application at 16 WAS,

G = additional P and K 40 kg/ha, respectively, in two times application at 16 and 18 WAS,

H = additional P and K 50 kg/ha, respectively, in one time application at 16 WAS

I = additional P and K 50 kg/ha, respectively, in two times application at 16 and 18 WAS,

Land preparation consisted of weed clearing, plowing and plots making. Plots were made with a width of 3 m and a length of 4 m. Plots distance was 0.3 m, while replications distance was 1 m. Plant spacing was 60 cm x 40 cm. Manure was given a week before planting at a dose of 2 tons/ha.

Seeds were mixed with fungicide, then two Job's tears seeds were planted in a hole with a depth of about 3 cm and covered by soil. Fertilizing of NPK at a dose of 200 kg/ha was given at 3 WAS by side dressing. The application of SP-36 and KCl fertilizer according to the treatment was given at 16 WAS (14 days after the flowers appeared) and 18 WAS (28 days after the flowers appeared) by side dressing.

Plant cultivation included weeding, pest and disease control. Weeds was controlled by mechanically weeding. Control of grasshopper was carried out by spraying insecticide, while fungicide controlled black mildew. Water supply for plant was given by surface irrigation.

Job's tears can be harvested at seed physiological mature, which is 165 days after sowing (DAS). Physically, dry and yellowish leaves indicated that seeds can be harvested. On the other hand, seeds having a shiny white color, filled or pithy, and hard when pressed by hand.

Observations were carried out by counting or measuring the components of yield, yield, and yield quality. The yield components consisted of the number of productive tillers, the number of panicles per tiller, the number of seeds per panicle, and the weight of 100 seeds. Yield were measured by seed weight per plant and harvest index, while yield quality were seed hardness, seed size, seed extraction ratio and polishing ratio.

The number of productive tillers, number of panicle, and the number of seed were counted at the age of 15 weeks after sowing. The number of productive tillers was counted based on the number of tillers that produce flowers at the reproductive stage. The number of panicle was

counted per tiller, while the number of seed was counted per panicle. Weight of 100 seeds and grain weight per plant was measured after harvest using an analytical scale at 14% moisture content. The harvest index is measured by comparing seed weight with plant biological weight. Seed size was measured using a caliper, while seed hardness was measured using a penetrometer. Seed extraction ratio was measured by dividing weight of hulled seed by weight of unhulled seed. Polishing ratio was measured by comparing the milled seeds with the Job's tears polished seed standard.

Data were analyzed using SPSS software version 25. Differences between treatments were identified using Scott-Knott test at 5% significance level.

Results and Discussion

Results. Addition of P and K fertilizers did not increase the number of productive tillers, the number of seeds, or the weight of 100 seeds of Job's tears plants compared to the control (Table 1). On the other hand, P and K fertilizer treatment at a dose of 30-40 kg/ha with a frequency of 1 or 2 times increased the number of panicles.

Crop yields can be increased by addition of P and K fertilizer (Table 2). Giving P and K fertilizers 20 or 30 kg/ha, either 1 or 2 times in the generative stage gave the best seed weight, while dose of 20 or 40 kg/ha that given 1 or 2 times and dose of 50 kg/ha that given 1 time application was better than control. The addition of P and K fertilizers at a dose of 50 kg that given twice did not increase yields compared to the control. The addition of P and K fertilizers at a dose of 20-40 kg/ha, either once or twice increased the harvest index compared to the control. The application of P and K fertilizers at a dose of 50 kg/ha which was given 1 time also increased yields, but the application 2 times gave the same results as the control.

In contrast to the yield and yield components, the addition of P and K fertilizers did not increase yield quality. The addition of P and K fertilizers did not give any difference in seed diameter, seed hardness, seed extraction ratio and polishing ratio compared to the control.

Table 1. The effect of addition of P and K fertilizers on the yield components of job's tears plants

Treatments	Number of Productive Tillers	Number of Panicles	Number of Seeds	Weight of 100 Seeds (g)
A = without additional P and K (control)	2.33 a	16.16 a	2.35 a	11.11 a
B = additional P and K 20 kg/ha, respectively, in one time application	4.00 a	13.11 a	2.39 a	12.60 a
C = additional P and K 20 kg/ha, respectively, in two times application	2.92 a	15.06 a	3.01 a	11.95 a
D = additional P and K 30 kg/ha, respectively, in one time application	3.42 a	18.23 b	2.74 a	12.12 a
E = additional P and K 30 kg/ha, respectively, in two times application	4.50 a	19.49 b	2.34 a	11.73 a
F = additional P and K 40 kg/ha, respectively, in one time application	2.50 a	16.50 b	2.94 a	11.76 a
G = additional P and K 40 kg/ha, respectively, in two times application	2.67 a	19.35 b	2.37 a	11.95 a
H = additional P and K 50 kg/ha, respectively, in one time application	3.33 a	13.44 a	2.99 a	12.26 a
I = additional P and K 50 kg/ha, respectively, in two times application	3.00 a	15.12 a	2.30 a	11.96 a

Note: The mean value of the treatment in the same column followed by the same letter was not different based on Scott-Knott test at the 5% significance level.

Table 2. The effect of addition of P and K fertilizers on the yield of job's tears plants

Treatments	Seed Weight per Plant (g)	Harvest Index
A = without additional P and K (control)	9.83 a	0.28 a
B = additional P and K 20 kg/ha, respectively, in one time application	15.79 b	0.34 b
C = additional P and K 20 kg/ha, respectively, in two times application	15.82 b	0.42 b
D = additional P and K 30 kg/ha, respectively, in one time application	20.70 c	0.41 b
E = additional P and K 30 kg/ha, respectively, in two times application	24.07 c	0.39 b
F = additional P and K 40 kg/ha, respectively, in one time application	14.26 b	0.42 b
G = additional P and K 40 kg/ha, respectively, in two times application	14.63 b	0.42 b
H = additional P and K 50 kg/ha, respectively, in one time application	16.41 b	0.46 b
I = additional P and K 50 kg/ha, respectively, in two times application	12.48 a	0.32 a

Note: The mean value of the treatment in the same column followed by the same letter was not different based on Scott-Knott test at the 5% significance level.

Table 3. The Effect of addition of P and K fertilizers on the yield quality of job's tears plants

Treatments	Seed Diameter (mm)	Seed Hardness (kgf)	Seed Extraction Ratio (%)	Polishing Ratio (%)
A = without additional P and K (control)	6.56 a	7.02 a	55.52 a	77.08 a
B = additional P and K 20 kg/ha, respectively, in one time application	6.68 a	7.09 a	56.25 a	79.50 a
C = additional P and K 20 kg/ha, respectively, in two times application	6.40 a	7.00 a	58.43 a	78.92 a
D = additional P and K 30 kg/ha, respectively, in one time application	6.47 a	6.90 a	60.03 a	79.33 a
E = additional P and K 30 kg/ha, respectively, in two times application	6.37 a	6.94 a	55.93 a	79.00 a
F = additional P and K 40 kg/ha, respectively, in one time application	6.33 a	7.04 a	54.95 a	79.58 a
G = additional P and K 40 kg/ha, respectively, in two times application	6.40 a	7.06 a	56.19 a	80.00 a
H = additional P and K 50 kg/ha, respectively, in one time application	6.61 a	6.99 a	57.15 a	79.42 a
I = additional P and K 50 kg/ha, respectively, in two times application	6.55 a	7.03 a	56.71 a	79.25 a

Note: The mean value of the treatment in the same column followed by the same letter was not different based on Scott-Knott test at the 5% significance level.

Discussion. Dry season that occurs during generative stage of plants (April – August) causes water shortages. The yield and yield components of Job's tears were smaller than previous studies which were conducted in sufficient water condition (Ruminta et al., 2017; Ruminta et al., 2018; Nurmala et al., 2016). Lack of water cause a decrease in phosphorus and potassium uptake (Ge et al., 2012; Bakhshandeh et al., 2020; Raza et al., 2012; Nawaz et al., 2012; Bista et al., 2018). This was shown by P and K treatments did not increase yield components or yield quality and only affected the number of panicles, seed weight, and harvest index. Nonetheless, there were several studies that state that potassium uptake in drought conditions actually increases (Raza et al., 2012). On the other hand, previous studies stated that the provision of phosphorus and potassium could increase yield components and yield quality (Chaichi et al., 2008; Malhotra et al., 2018; Seneweera & Conroy, 1997; Brennan & Bolland, 2009; Ali et al., 2003).

Adding phosphorus and potassium fertilizer 30-40 kg/ha increased the number of panicles, either in one or two applications. The data showed that the dose affected more than the frequency of fertilizer application. Increasing the dose of phosphorus has been known to

increase the number of flowers (Vosnjak et al., 2021; Malhotra et al., 2018). The same thing happened for increasing the dose of potassium (Fageria & Oliveira, 2014; Fageria, 2015). However, giving too much phosphorus and potassium (50 kg/ha) did not increase the number of panicles (Islam & Muttaleb, 2016; Banerjee et al., 2018).

On crop yield, the best doses of phosphorus and potassium were narrowed at 30 kg/ha, either 1 or 2 applications. The analogy in the number of panicles observation, an increase in the doses of phosphorus and potassium increased yield, but the provision of too many doses of phosphorus and potassium did not increase the yield. In this case, the number of panicles as a yield component increased due to the application of phosphorus and potassium, causing an increase in plant yield. Increasing a yield component could result in the increasing of yield (O'Connor et al., 2018; Huang et al., 2011). The interesting thing was that twice application of 50 kg P and K fertilizer did not increase yields, compared to one time application. This was inconsistent compared to the same frequency of fertilization at other fertilizer doses.

The harvest index is affected by crop yield (Wnuk et al., 2013; Li et al., 2015). With an increase in seed weight due to the provision of

phosphorus and potassium, the harvest index also increased compared to the control. However, the dose of 30 kg/ha, which gave the best seed weight, did not increase harvest index compared to doses of 20, 40, or 50 kg/ha. This probably happens because the provision of phosphorus and potassium can increase biological weight (Dai et al., 2016; Bilal et al., 2021; Maurya et al., 2014).

Conclusions

The addition of phosphorus and potassium in the generative stage of Job's tears increased the number of panicles, seed weight, and harvest index but did not affect other yield components and yield quality.

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Kustanto H

Testing of resistance to downy mildew on several sweet corn inbred lines of 7th generation

Abstract. Sweet corn (*Zea mays saccharata* Sturt) is one of the most well-known horticultural crop commodities and can adapt to various climates and environments. Efforts to control downy mildew disease continue to be carried out in collaboration with various parties in different ways, including genetic improvement of the varieties planted. The purpose of this study was to test the resistance of the 7th generation of sweet corn inbred lines to downy mildew and to find out the phenotypic characters that affect downy mildew. The new inbred lines obtained from the results of a long breeding process will be better tested for resistance to downy mildew before crosses are carried out among these inbred lines to guarantee better the development of new varieties of sweet corn that are resistant to downy mildew. The experiment was conducted from August to November 2019 in Getasrejo, Grobogan, and Central Java. The material consisted of 10 kinds of maize inbred line seeds and two comparison varieties. The experiment was carried out using a randomized block design with three replications. One of the obstacles that is difficult to overcome in sweet corn cultivation is downy mildew disease (*Peronosclerospora spp.*). The conclusions of this study were: (1) Inbred lines HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04, and HSJM 01 were categorized as highly resistant genotypes. HSJM 09 can be categorized as a resistant inbred line. Inbred lines HSJM 10 and HSJM 03 were classified as moderately resistant genotypes. (2) The characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining selection criteria against downy mildew disease on sweet corn.

Keywords: Attack · Downy mildew · Resistance · Sweet corn

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Kustanto H^{1*}

¹ Faculty of Agriculture, Universitas Wahid Hasyim, Jl. Menoreh Tengah X/22, Sampangan, Gajahmungkur, Semarang 50232, Indonesia

*Correspondence: heri.kustanto@unwahas.ac.id

Introduction

Sweet corn (*Zea mays saccharata*) is one of the best-known horticultural commodities. Sweet corn could adapt to various climates and environments so that it could be bred easily by farmers. The sweet corn seeds contain solid components (27.3%) and water (72.7%). The solid parts of the sweet corn seed contain hydrocarbons (81%), protein (13%), fat (3.5%), and other materials (2.5%) (Alfandri et al., 2014; Swapna et al., 2020). Also, sweet corn seeds contain vitamins that benefit the human body (Szymanek, 2012).

As is the case with forage corn, breeding sweet corn faces many obstacles, including the onset of downy mildew (*Peronosclerospora spp.*). Downy mildew is the main disease that reduces sweet corn productivity. Today, it is reported that downy mildew has infected new superior varieties of various corn types at the start of the growth phase (Daryono et al., 2018). In addition to the severe infestation, the disease always exists, is latent, and threatens the production of sweet corn (Daryono et al., 2018; Prasetyo et al., 2021). The causes of latent downy mildew infestation are the presence of early inoculum sources, asynchronous corn planting patterns, and the application of susceptible varieties (Hendrayana et al., 2020; Prasetyo et al., 2021).

Efforts to control downy mildew continue to be carried out in collaboration with various parties. Downy mildew can be controlled through crop rotation, eradication, seed treatment, organic and inorganic pesticides, and the use of resistant superior varieties (Alfandri et al., 2014; Mariani et al., 2019; Pajrin et al., 2013; Sonhaji et al., 2013). Efforts to control the disease with superior varieties are made through the breeding process. The conventional breeding process was started by preparing basic population material, the inbred line, and combining ability selection and resistance selection to biotic and abiotic factors. A method of breeding resistance to biotic disturbance is testing the inbred lines against resistance to downy mildew infestation (Ali et al., 2019; Daryono et al., 2018; Kustanto et al., 2012).

It is expected that the selection of lines that are resistant to downy mildew will be able to support the hybrid sweet corn assembly, which is more resistant to downy mildew, so that it can be produced easily and economically (Daryono et al., 2018; Janruang & Unartngam, 2018). The new

inbred lines, which were derived from a long breeding process, are better tested for resistance to downy mildew before being crossed between inbred lines. The objectives of the study were to test the resistance of the sweet corn inbred lines of the 7th generation to downy mildew and to find out the phenotypic characteristics of the corn that relate to downy mildew disease to facilitate the determination of selection criteria. By knowing the lines that are resistant to downy mildew, it is expected that new varieties of sweet corn that are more resistant to downy mildew will be produced.

Materials and Methods

The planting materials comprised 10 sweet corn inbred lines of the 7th generation that had been developed by CV. Hakako Seed. The inbred lines were: HSJM 01, HSJM 02, HSJM 03, HSJM 04, HSJM 05, HSJM 06, HSJM 07, HSJM 08, HSJM 09, and HSJM 10, along with 2 comparison varieties, namely VP 01 and VP 02. VP 01 is a commercial sweet corn variety that has high production and better resistance to downy mildew, while VP 02 is a forage corn variety that has high production but is highly susceptible to downy mildew. Other materials were NPK at a dose of 300 kg/ha and Urea at a dose of 50 kg/ha. The devices used were dibbles, meters, Crown brand manual scales, and hand counters. The experiment was conducted from August to November 2019 in Getasrejo, Grobogan, and Central Java. The experiment was carried out using a randomized block design with three replications. The spacing was 70 x 20 cm, and the population per plot was 100 plants.

Planting was done using two seeds per planting hold. Thinning was carried out at 10 days after planting (DAP) to obtain an appropriate population of 100 plants per plot. The fertilization doses were Phonska 300 kg/ha and Urea 250 kg/ha. The first fertilization was applied at 10 DAP using phonska at 300 kg/ha. The second fertilization was applied at 21 DAP using urea at 200 kg/ha, and the third was applied at 35 DAP using urea at 50 kg/ha. The Inoculum was prepared three weeks before the experiment was carried out. The susceptible varieties (*spreader row*) were planted in 4 rows around the replication plot and 4 rows between each replication. After the *spreader row infection* was found to be 20-30%, it was followed by experimental planting. To strengthen the

infection, conidia, which were dissolved in sugar solutions, were sprayed on leaves that were infected by downy mildew. And then, the solutions were sprayed on the experiment field when the plants were 10 and 12-days old at 3-4 a.m. in the morning (Hendrayana et al., 2020; Kim et al., 2016).

Some characters that being observed were: (a) leaf width (cm), measured from outside of the right side and the left side (cm), (b) leaf length (cm), measured from the base to the tip of the leaf (cm), (c) leaf wet weight (g), by weighing the wet weight of freshly picked leaves, (c) leaf dry weight, by weighing the leaf dry weight after sun-dried for 3 days until the leaves crumble when crushed, (d) moisture content (%) of the leaves is calculated by the formula: $MC = (DW - WW) \times 100\%$, in which MC = moisture content, DW = leaf dry weight, WW = leaf wet weight, (d) number of leaves (pcs), calculate number of leaves from the base to the tip of the flag leaf, (e) plant height (cm), calculate the plant height from the ground level to the base of the flag leaf. Observation on the intensity of the downy mildew infestation was conducted at 14, 21, 28, 35, and 42 DAP. The percentage of the downy mildew infestation was calculated by the formula: $I = (A/B) \times 100\%$. In which: I= percentage of the downy mildew infestation, A= number of plants infected by downy mildew, and B= number of plants observed in each tested genotype. Criteria for plant resistance are as follows: (a) Highly Resistant (HR) is the disease onset 0 - 10%, (b) Resistant (R) is the disease onset 10 - 20%, (c) Moderately Resistant (MR) is the disease onset 20 - 40%, (d) Susceptible (S) is the disease onset 40 - 60% and Highly Susceptible (HS) is the disease onset 60 - 100% (Daryono et al., 2018; Khoiri et al., 2021).

Data processing used Anova at level 5%. Post-Hoc Test was carried out to determine the effect of significant difference treatment using Duncan Multiple Range Test (DMRT) at level 5%. Relationship among the observed variables was analyzed with correlation by the formula: $rp = (Cov_{xy}) / (\sqrt{\sigma^2x \cdot \sigma^2y})$. Where: rp = coefficient of correlation, Cov_{xy} = trait covariance x and y, σ^2x = trait variance x, dan σ^2y = trait variance y (Singh & Chaudary, 1998; Kustanto, 2022). Calculation for the correlation values are: (a) not strong (0.1 - 0.2), (b) rather strong (0.2 - 0.4), (c) strong enough (0.4 - 0.6), (d) strong (0.6 - 0.8) and (e) very strong (0.8 - 1.0).

Results and Discussions

Performance of the Vegetative Characters.

Results for the combined analysis of variance showed significant influence on characters of leaf width, leaf wet weight, leaf dry weight, leaf moisture content, plant height, and infestation level of downy mildew (*Peronosclerospora* spp). Results for the combined analysis of variance on characters of leaf length and number of leaves per plant did not show any significant influence. Results for analysis of variance, coefficient of variance, and influence of the significantly different treatments with Duncan's Multiple Range Test are presented in Table 1.

Results for the combined analysis of variance toward leaf length showed significant influence among the tested genotypes. Leaf length in all treatments ranged 78.73 - 91.33 cm. HSJM 08 had the lowest leaf length, 78.73 cm, while HSJM 04, HSJM 03, and VP 02 had the highest leaf lengths 91.33, 90.40, and 90.00 cm, respectively, and were not significantly different with HJSM 01, HSJM 02, HSJM05, HSJM 09 and VP 01. Leaf width did not show significant difference among the tested genotypes. In comparison with the character of leaf width, leaf length showed variations on the tested genotypes. Inbred lines of corn showed different appearances in leaf sizes, which are different from the comparative varieties, the hybrids. Sari and Hafsa (2022) reported that crop varieties have a specific characteristic that can be set as superiority value of a given variety. Factors which affect different character in each line can be affected by genetic factors from heredity.

Results for the combined analysis of variance on leaf wet weight showed significant influence among the tested genotypes. Leaf wet weight ranged 12.40 - 16.04 g. The lowest leaf wet weight was 12.40 g for HSJM 10 and it was not significantly different from HSJM 06, HSJM 07, HSJM 08, VP 01 and VP 02. The highest wet weight was 16.04 for HSJM 02 and it was not significantly different from HSJM 01, HSJM 03, HSJM 05, HSJM 08 and HSJM 09. Results for the combined analysis of variance toward leaf dry weight showed significant influence among the tested genotypes. The leaf dry weight ranged from 3.07 - 4.34 g. The lowest leaf dry weight was 3.07 g for VP 02 and it was not significantly different from HSJM 10 and VP 01. The highest leaf dry weight was 4.34 g for HSJM 02 and it was

not significantly different from HSJM 01, HSJM 03, HSJM 05, HSJM 08 and HSJM 09. Results for the combined analysis of variance toward moisture content in leaf showed significant influence among the tested genotypes. The leaf dry weight ranged from 24.52 – 29.60 g. The lowest moisture content was found on HSJM 07 and HSJM 08, 24.52 and 24.80 g, respectively, and they were not significantly different from HSJM 04, HSJM 05, HSJM 06, HSJM 09 and VP 02. The highest moisture content in leaf was 29.60 on HSJM 02 and was not significantly different from HSJM 01, HSJM 03, HSJM 04, HSJM 06, HSJM 10, VP 01 and VP 02. Characters of leaf wet weight, leaf dry weight and moisture content in leaf showed many variations among the tested genotypes. Not only differences among lines, characters of leaf wet weight, leaf dry weight, and moisture content of leaf also have obvious and significant differences compared to the hybrid variety as the comparison. The growth process is affected by internal and environmental factors. The internal factor is the used genotypes, and the environmental factors are temperature, light, water, and etc. (Mariani et.al. 2019).

Number of leaves per plant did not show any significant difference and ranged 12.20 – 13.30. Results for the combined analysis of variance toward plant height showed significant influence among the tested genotypes. The plant heights ranged 160.40 – 185.40 cm. The lowest plant heights were found on HSJM 02, HSJM 04, HSJM 10 for about 160.63, 161.97, and 160.40 cm, respectively, and were not significantly different from HSJM 03, HSJM 06, HSJM 09, VP 01 and VP 02. The highest plant heights were found on HSJM 01, HSJM 05, HSJM 07 and HSJM 08 for about 195.40, 180.07, 180.4, 180.30 cm, respectively, and were not significantly different from HSJM 03, HSJM 06, HSJM 09, VP 01 and VP 03. Plant heights showed a difference among the tested genotypes. The tested inbred lines of the sweet corns showed high variations among the tested genotypes on character of the plant height. The inbred lines have a homozygous genetic composition and tend to have weak adaptation to the environment (Ali et al., 2019). The hybrid varieties have heterosis effects and high vigor and can adapt well to less supportive environments than their parents (Agustiani et al., 2019; Xiao et al., 2021).

Table 1. Characters of vegetative components of the tested genotypes

Genotype	LL		LW		LWW		LDW		LM		NLP		PH	
HSJM 01	88.17	bcd	8.23	14.71	bcde	3.95	cde	28.59	bc	12.63	185.40	b		
HSJM 02	89.30	cd	7.87	16.04	e	4.34	e	29.60	c	13.30	160.63	a		
HSJM 03	91.33	d	8.67	15.21	cde	4.11	de	28.80	bc	12.83	170.40	ab		
HSJM 04	90.40	d	7.80	12.91	abc	3.68	bcd	27.06	abc	13.27	161.97	a		
HSJM 05	83.70	abcd	8.17	14.67	bcde	3.65	bcd	25.67	ab	13.23	180.07	b		
HSJM 06	81.03	abc	8.13	12.92	abc	3.63	bcd	26.33	abc	12.23	175.50	ab		
HSJM 07	80.57	ab	8.13	13.17	abcd	3.70	bcd	24.52	a	12.37	180.40	b		
HSJM 08	78.73	a	8.13	14.00	abcde	4.07	de	24.80	a	12.40	180.30	b		
HSJM 09	84.93	abcd	8.00	15.61	de	4.10	de	25.62	ab	12.97	174.47	ab		
HSJM 10	85.60	abcd	8.73	12.40	a	3.43	abc	28.88	bc	12.60	160.40	a		
VP 01	85.27	abcd	8.17	13.37	abcd	3.37	ab	28.37	bc	12.20	170.50	ab		
VP 02	90.00	d	8.77	12.73	ab	3.07	a	26.73	abc	12.87	173.90	ab		
Mean	85.75		8.23	13.98		3.76		27.08		12.74	172.83			
CV (%)	5.34		8.19	9.34		8.16		6.61		6.79	5.33			
Sign.	*		ns	**		**		*		ns	*			

Notes: LW: Leaf Width, LL: Leaf Length, LWW: Leaf Wet Weight, LDW: Leaf Dry Weight, LM: Leaf Moisture, NLP: Number of Leaves per Plant, PH: Plant Height.

Resistance to Downy Mildew. Results for the combined analysis of variance toward percentage of the downy mildew infestation showed significant influence among the tested genotypes. Percentage of the downy mildew onset ranged 2.67 – 50.00%, as presented in Table 2. The lowest percentage of the downy mildew infestation was 2.67% on HSJM 08 and it was not significantly different from HSJM 06, HSJM 07 and HSJM 02 that were 3.33%, 3.33% and 6.00%, respectively. The highest percentage of downy mildew infestation was 50% on the comparison variety 02. Percentage of the downy mildew infestation was highly varied from 7, 14, 21, 27 and 28 DAP and showed high coefficient of variances that ranged 36.60 – 46.10%. The incubation periods were varied due to some factors, for instance, pathogen virulence, host resistance, and environmental conditions, such as temperature and humidity that support the pathogen development (Ulhaq and Masnilah, 2019). Percentage of the downy mildew infestation at the age of 34 and 42-day old tended to be stable with lower coefficient of variances, 17.13 – 18.43%. According to the criteria, the comparison genotype of VP 02 is the inbred line which is susceptible, while the inbred lines of HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04 and HSJM 01 are characterized as highly resistant genotypes. HSJM 09 is

characterized as resistant inbred line. While the inbred lines of HSJM 10, HSJM 03 and VP 01 are categorized as moderately resistant.

This fact showed that the tested sweet corn inbred lines had good resistant to downy mildew and superior. Inbred lines that have good resistant to downy mildew can be used as the parent in the hybrid varieties assembly (Daryono et al., 2018; Khoiri et al., 2021). Besides the genetic factor, the environmental factors also affect severity of the downy mildew infestation. The genetic resistance is brought about by heredity factor and also it is presumed to be affected by the morphological factors (Ulhaq and Masnilah, 2019). The environmental factors are temperature, humidity, and wind direction. Wind velocity and location of the inoculum determine the occurrence of downy mildew epidemics, while the wind direction affects the infection rate on sweet corn (Purwanto et al., 2016).

Values for coefficient of variance at 7 DAP, 14 DAP, 21 DAP, 27 DAP, and 28 DAP were very high, while at 34 DAP and 42 DAP were medium. The fact might be caused by different incubation periods among genotypes. The incubation period is the time between the onset of infection and the onset of symptoms. The crop which resistant to a disease will indicate longer incubation period (Ulhaq and Masnilah, 2019)

Table 2. Characters of the infestation percentage and categories of the downy mildew infestation on the tested genotypes.

Genotype	% infestation of downy mildew							Category
	7 DAP	14 DAP	21 DAP	27 DAP	28 DAP	34 DAP	42 DAP	
HSJM 01	1.00 ab	1.00 ab	4.00 ab	1.67 a	7.33 abcd	8.00 c	9.00 cd	HR
HSJM 02	1.00 ab	1.00 ab	1.33 a	11.67 abc	4.67 abc	6.00 bc	6.00 abc	HR
HSJM 03	5.33 ab	5.33 ab	7.00 abc	2.33 a	19.00 ef	33.00 f	34.33 f	MR
HSJM 04	1.33 ab	1.33 ab	1.33 a	3.67 ab	4.00 ab	6.33 bc	7.33 bc	HR
HSJM 05	1.67 ab	1.67 ab	3.00 ab	1.00 a	4.00 ab	6.00 bc	8.00 c	HR
HSJM 06	0.00 a	0.00 a	0.33 a	1.33 a	1.33 a	2.67 ab	3.33 ab	HR
HSJM 07	1.00 ab	1.00 ab	1.00 a	1.00 a	1.67 a	2.33 ab	3.33 ab	HR
HSJM 08	0.67 ab	0.67 ab	0.67 a	12.00 abc	1.67 a	1.67 a	2.67 a	HR
HSJM 09	6.33 ab	6.33 ab	10.67 bc	11.00 abc	14.33 cde	15.33 d	13.67 d	R
HSJM 10	7.33 b	7.33 ab	8.67 abc	8.67 abc	13.00 cde	20.33 e	22.00 e	MR
VP 01	3.67 ab	3.67 ab	4.67 abc	17.67 c	12.33 bcde	19.00 de	22.67 e	MR
VP 02	8.67 b	8.67 b	13.33 c	6.41	24.33 f	42.67 g	50.00 g	S
Mean	3.17	3.17	4.67	6.42	8.97	13.61	15.19	
CV (%)	46.10	46.07	36.60	40.96	28.34	18.43	17.13	

Notes: %: percentage, DAP: day after planting, HR: Highly Resistant, MR: Moderately Resistant, R: Resistant and S: Susceptible

Correlation among the observed traits.

Correlation between the observed characters and percentage of the downy mildew infestation showed varying outcomes. The character of leaf width showed strong correlation with percentage of downy mildew infestation and has positive value (0.57). Positive correlation refers to an increase in one trait followed by an increase in another trait. On the contrary, negative correlation refers to an increase in one trait will be followed by a decrease in other trait (Pudjiwati et al., 2013; Novrika et al., 2016; Sudjana, 1992).

The character of leaf wet weight. Leaf length had strong correlation with percentage of downy mildew infestation and had positive value. Wet weight did not correlate with percentage of the downy mildew infestation. Leaf dry weight correlated with percentage of downy mildew infestation and had negative value. Leaf moisture correlated rather strongly with percentage of the downy mildew infestation. Number of leaves per plant and plant height did not correlate with percentage of the downy mildew infestation, and these are presented in Table 3.

Correlation among characters showed relationship among values obtained by each character. Correlation among characters may has positive or negative values. Positive value means that the character is directly proportional to the change in value. While the negative correlation means that the character is inversely

proportional to the change in value (Sudjana, 1992; Kustanto, 2023). Characters of leaf width and leaf length correlated with percentage of the downy mildew infestation in which the increase in sizes of the leaf width and leaf length can increase percentage of the downy mildew infestation on the sweet corn. Leaf wet weight did not affect the percentage of downy mildew infestation. Leaf dry weight moderately affected the percentage of downy mildew, which the increase of leaf dry weight will reduce the percentage of downy mildew infestation. Moisture content in leaf rather strongly affected the percentage of downy mildew infestation, in which the higher the moisture content in leaf will increase the infestation of downy mildew. Number of leaves per plant and plant height did not affect the percentage of downy mildew infestation. Characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining the selection criteria against downy mildew infestation on sweet corn. The production of sweet corn is affected by the production variable. In general, the influence of variety on production is in line with the effect of variety on disease intensity, in which the lower intensity of the disease, the higher yield will be produced (Ginting et al. 2020). Results of the research showed that cytoplasm constitution of the female parent affects the inheritance of downy mildew in the hybrids (Elmoghazy et al, 2018).

Table 3. Correlation among the observed variables

	LW	LL	LWW	LDW	LM	NLP	PH	% DMI
LW	1							
LL	0.23	1						
LWW	0.22	-0.32	1					
LDW	0.00	-0.48*	0.83**	1				
LM	0.71**	0.24	0.20	0.10	1			
NLP	0.60**	-0.23	0.47*	0.29	0.20	1		
PH	-0.49*	0.00	0.10	0.02	-0.58**	-0.38*	1	
% DMI	0.57**	0.79**	-0.18	-0.51	0.32	0.05	-0.19	1

Notes: LW: Leaf Width, LL: Leaf Length, LWW: Leaf Wet Weight, LDW: Leaf Dry Weight, LM: Leaf Moisture, NLP: Number of Leaves per Plant, PH: Plant Height, % DMI: Percentage of Downy Mildew Infestation.

Conclusions

Based on results of the research, some conclusions are drawn as follow:

1. Inbred lines of HSJM 08, HSJM 06, HSJM 07, HSJM 02, HSJM 05, HSJM 04, and HSJM 01 are categorized as highly resistant genotypes. HSJM 09 is categorized as resistant inbred line. HSJM 10 and HSJM 03 inbred lines are categorized as moderately resistant genotypes.
2. Characters of leaf width, leaf length, leaf wet weight, leaf dry weight, and leaf moisture content can be considered in determining the selection criteria against downy mildew infestation on sweet corn.

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Widarawati R · Syarifah RNK · Prakoso B

Leaf character of sugar palm on various shades and concentrations of liquid organic fertilizer

Abstract. Palm sugar has been developed to produce various products and raw materials for their derivatives. There is a very high morphological diversity of sugar palm plants (*Arenga pinnata* (Wurmb) Merr.), while the morphological identification of different sugar palm plant species in Banten province is still limited. The aim of this research is to determine the effect of different treatments, such as shade percentage, liquid organic fertilizer concentration, and a combination of both, on the properties of palm leaves. The experimental design used in this study was the 2-factor RCBD split plot with 3 levels each. The first factor is the variation of the shadow percentage, namely N0 (no shadow), N1 (55% shadow), and N2 (85% shadow). The second factor is different concentrations of liquid organic fertilizer (POC), namely P0 (0 ml/liter water), P1 (1 ml/liter water), and P2 (2 ml/liter water). The results showed that different types of shade yielded insignificant results in terms of variable number of leaves, total leaf area, and leaf greenness. The different types of liquid organic fertilizer concentration treatments and treatment combinations of shade percentage and liquid organic fertilizer concentrations did not influence any of the observed variables.

Keywords: Growth · Palm · Organic fertilizer · Shade

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Widarawati R¹ · Syarifah RNK^{1*} · Prakoso B²

¹ Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Universitas Jenderal Soedirman,
Jl. Dr. Soeparno No.63, Karang Bawang, Purwokerto Utara, Banyumas 53122, Indonesia

² Laboratory of Plant Protection, Faculty of Agriculture, Universitas Jenderal Soedirman,
Jl. Dr. Soeparno No.63, Karang Bawang, Purwokerto Utara, Banyumas 53122, Indonesia

*Correspondence: risqanaila@unsoed.ac.id

Introduction

Sugar palm (*Arenga pinnata* Merr) is one of the important palm plants after coconut and oil palm. Almost all parts of this annual plant have economic value and can be utilized, so it has a high potential to be developed (Sebayang, 2016).

The main problem with sugar palm plants is that the intensive cultivation system has not been carried out. Sugar palms are usually allowed to grow naturally without advanced treatment, so the sap yield is still relatively low. Therefore, it is necessary to carry out various treatments for sugar palm plants that can increase production (Paulina et al., 2018; Rattan et al., 2022).

Shading can be one of the attempts to adjust the light intensity to the needs of the seedlings and is expected to have a positive effect on the growth of the seedlings. The sugar palm naturally grows in the shade of the canopy in the country, so the level of shade is one of the most important factors influencing the growth of the sugar palm. Research by Furqoni et al. (2018) shows that shady palm seeds have more optimal growth. 56% shade can increase the wet weight, dry weight, and root volume of sugar palm seedlings compared to seeds that receive no shade. According to Samal et al. (2020), the sugar palm plant is a semi-shade tolerant plant, which means that the sugar palm needs shade early in its growth. According to Barus (2017), young oil palm seedlings need shade to reduce the intensity of sunlight at the beginning of growth because too high or too low light intensity is one of the inhibiting factors for the growth of young oil palm seedlings. There is limited information on the optimal amount of shade for sugar palm farms, so it is necessary to conduct research. In addition to shading, the carrying capacity of the soil is an important factor in the cultivation phase. The low availability of nutrients in the soil for seedlings can be overcome by adding nutrients through organic fertilizers.

Liquid organic fertilizer is widely used to add nutrients to plants from organic waste processing (Roidah, 2013). The liquid organic fertilizer content used in this study includes 0.18% N+P₂O₅+K₂O total, 4.6% C Organic, 41.04 ppm Zn, 8.43 ppm Cu, 2.42 ppm Mn, 2.54 ppm Co, 0.45 ppm Fe, 0.12% S, 60.40 ppm Ca, 16.88 ppm Mg, 0.29% Cl, 0.15% Na, 60.84 ppm B, 0.01% Si, 6.38 ppm Al, 0.98% NaCl, 0.11 ppm Se, <0.06 ppm Cr, <0.2 ppm Mo, <0.04 ppm V, 0.35% So₄, pH 7.91, 0.44% lipid, 0.72% proteins, 0.01%

humic, vulvic, gibberellin, cytokinin, auxin (Stockistnasa, 2022).

Leaves have a special function in photosynthesis and respiration with a flat shape, which makes it easy to receive sunlight and carbon dioxide and facilitate the process of water and water vapor flowing in and out. Morphologically, leaves have different shapes, colors, sizes, and textures. This is related to the task of these organs to carry out the basic physiological functions of plants. Conversely, leaves are structurally divided into the epidermis (with stomata), mesophyll, and transport tissue. However, the composition of these components is influenced by the physical environment, such as water availability, light intensity, and the ecological niches of the plants (Riyaldi et al., 2017).

The aims of this study were (1) to determine the effect of various shading on the characteristics of palm leaves, (2) to determine the effect of different liquid organic fertilizer concentrations on the characteristics of palm leaves, and (3) to know the effect of the combination of organic liquid fertilizer concentration and the best shade for the characteristics of palm leaves.

Materials and Methods

The research was conducted at the screen house of the Experimental Farm of the Faculty of Agriculture, Universitas Jenderal Soedirman, with an altitude of 110 meters above sea level. The study was carried out from September until December 2021. The experimental design used in this study was the Split Plot Randomized Block Design with 2 factors with 3 levels each. The first factor is the percentage of shade, namely N₀ (without shade), N₁ (55%), N₂ (85% shade). The second factor is the type of liquid organic fertilizer concentration, namely P₀ (without fertilizer), P₁ (1 mL liter⁻¹), P₂ (2 mL liter⁻¹).

Shade was applied at the beginning of the study with the respective percentages of 55% and 85% ± 2m from the top of the seedling and ±0.5m to the side of the seedling. The application of liquid organic fertilizer was carried out 8 times with doses according to treatment, as much for every plant once every 14 days by pouring it into polybags. Data collection for sugar palm plants was carried out when the plants were 20, 40, 60, and 80 days after transplanting.

Observed variables include:

1. Increasing the number of leaves (sheets)
Fully opened leaves are counted. The increase in leaf number was measured at 40 and 80 days of age after transplanting and measured by the formula (Farida, 2017):
The increase in the number of leaves = [the number of last leaves - the number of early leaves].
2. Total leaf area (cm²)
The total leaf area is calculated using the length per width method using the following formula:
Leaf area = $P \times W \times k$,
where LD = leaf area; P = leaf length; L = blade width; and k = (0.66) constant.
The value of this constant is obtained by comparing the value between the actual leaf area (which was performed using the graph paper method in this study) and the estimated leaf area using the length x width value. After transplanting, the total leaf area was measured at 40 and 80 days of age (Susilo, 2015).
3. Stomata density (µm²)
Stomata density is a comparison of the number of stomata in the area (Marantika, 2021) and is measured at 40- and 80-days post-transplantation (HPST). Calculated with the formula:
Stomata density = (number of stomata)/(field of view)
The formula calculates the field of view: $1/4 \times \pi \times d^2$, $\pi = 3.14$, and d = diameter of the microscope's field of view = 0.45.
4. Green of Leaves (unit)
Chlorophyll content was measured on the plant's top, middle, and bottom leaves using the Soil Plant Analysis Development (SPAD) tool. This measurement is made on the 2nd wing of the last fully opened wing. The increase in the number of leaves was measured at 40 and 80 days after transplanting.

The results of the observation of variables consisting of the increase in the number of leaves, total leaf area, stomata density, and green leaves were then analyzed for variance with the F test at the 5% level that had a significant effect. It was continued with the Duncan's Multiple Range Test (DMRT) tests at the significant level of 5% at the DSAASTAT application.

Results and Discussion

The analysis using the F test showed that the type of shade treatment only affected the increase in

the number of leaves, the total leaf area, and the greenness of the leaves (Table 1).

Table 1. The average data on the effect of various percentages of shade and types of liquid organic fertilizer concentrations on the leaves of sugar palm seedlings (5 months old).

Treatments	INL (sheet)	TLA (cm ²)	SD (µm ²)	GL (unit)
Shade	7.142	5.183	3.170	9.375
F 0.5	5.143	5.143	5.143	5.143
Sig.	*	*	ns	*
Liquid organic fertilizer	2.689	1.023	0.289	2.514
F 0.5	3.555	3.555	3.555	3.555
Sig.	ns	ns	ns	ns
Shade X Liquid organic fertilizer	0.61	0.213	0.481	2.758
F 0.5	2.928	2.928	2.928	2.928
Sig.	ns	ns	ns	ns

Note: INL = increase of number leaves, TLA = total leaf area, SD = stomata density, GL = greenish leaves. The numbers listed are obtained from the results of the analysis of variance using the F test at the 5% level. Ns = not significantly different, * = significantly different at Duncan's test.

Increase the number of Leaves. The significant difference in the variable number of leaves in different types of percent tint treatment shows no significant difference in the treatment of different organic liquid fertilizer concentrations and their combinations (Table 1). Based on the results of further tests on the effect of shade types, the highest number of leaves was without shade, with 12.58 leaves, and the smallest number of leaves was found in the 55% shade, with 8.42 leaves (Figure 1).

The results showed that sugar palm seedlings with higher light intensity without shade had more leaves than sugar palm seedlings with 55% & 85% shade. The lower the light intensity, the fewer leaves (Akmalia & Suharyanto, 2017). Low light intensity will cause the ambient temperature to decrease and affect the primordial temperature, delaying the initiation of leaf formation. The number of leaves affected by shade has fewer leaves than plants that get full compared to those without shade light. The number of leaves affected by shade has fewer leaves than plants that get full light (without shade) (Handriawan et al., 2016; Wahba et al., 2016).

Balanced fertilization has a very positive effect on plant growth. The higher the concentration of

liquid organic fertilizer used, the greater its contribution in providing the nutrients needed in plant physiological processes, but the balance of the availability of nutrients absorbed by plants will determine the increase in plant growth (Ramírez et al., 2010; Oktaviani et al., 2022).

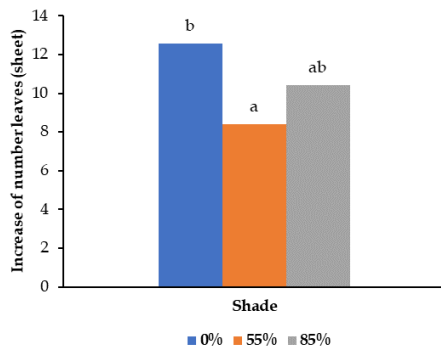


Figure 1. Bar chart of the increase in the number of sugar palm leaves.

Total Leaf Area. Various treatments of shade percentage to the total leaf area showed significantly different results. The F-count value is lower than the F-table, so H_0 is rejected. After further testing with the DMRT methods at the 5% level, there is no difference in letter notation (Figure 2).

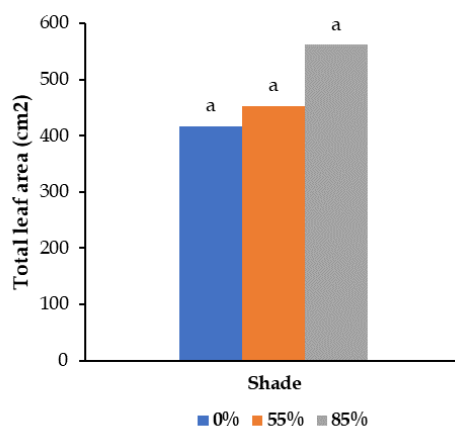


Figure 2. Bar chart of total leaf area on sugar palm plants.

Treatment of shade ratio to leaf area showed no difference between treatments. The percent shade with total leaf area values at 0.55% and 85% treatment was 416.56 cm²; 418.50 cm² and 562.06 cm². This may be due to the influence of various existing environmental factors, such as the condition of the palm seeds in the greenhouse, which may have a different exposure to sunlight

due to the location of the research greenhouse between other greenhouse buildings. In addition, changing environmental conditions, both in terms of temperature, humidity, and intensity of sunlight, can prevent the treatment from having a different effect. Apart from the fact that plants are generally capable of photosynthesis under optimal lighting conditions, the large increase in leaf area may also be due to plants adapting to the increase in light-trapping area, allowing photosynthesis to proceed normally even under shady conditions (Supriyono et al., 2017). An increase in leaf area in shaded plants is a mechanism of plant adaptation to shade stress (Xie et al., 2020; Ren et al., 2022). Extend these efforts to increase the light capture range for more efficient photosynthesis.

The treatment of liquid organic fertilizer concentration and its combination with shade had no significant effect on the total leaf area, one of which could be due to the incompatibility of the nutrient requirements of sugar palm seeds with the Liquid organic fertilizer concentration given. Several things must be considered in the application of liquid organic fertilizer, namely the type of liquid fertilizer used, the nutrient content, the concentration of the solution, and the time of application (Ren et al., 2022; Fernández-Delgado et al., 2022). Nutrient requirements in plants for growth and development are not the same, require different times, and not the same amount. Fertilization treatment should be given when plants need nutrients intensively for growth and development to take place properly.

Stomata Density. Variable stomata density, given various treatments such as percentage of shade, kinds of organic liquid fertilizer concentrations, and a combination of different percentages of shade and liquid organic fertilizer concentrations, showed results that were not significantly different in the analysis using the F test. The F-count value of each treatment is lower than the F-table (Table 1). This indicates a high value for each shading treatment for all variables.

These results showed that sugar palm seedlings treated without shade had higher light intensity and stomata density than palm seeds with shade (85%), which received lower light intensity. The number of leaf stomata in the treatment of high light intensity (without shade) was more than in the shade treatment, and shade reduced the density or distribution of stomata (Fitriatin et al., 2019). The absorption of nutrients that enter through the stomata will be different

and have a very small effect on plant growth and yield if the time of spraying liquid fertilizer is different. It is not recommended to spray foliar fertilizer when the air temperature is hot because the concentration of the fertilizer solution that reaches the leaves quickly increases so that the leaves quickly increase so that the leaves can burn (Bergstrand, 2022)

Greenish Leaves. Various treatments of the percentage of shade on the greenness of the leaves gave significantly different results. Based on Table 1, which means the percentage of shade is significantly different. Further test results in Table 1. also show a significant difference according to the DMRT at the 5% level. The best type of shade treatment was 85% shade with a greenish value of 48.48 units.

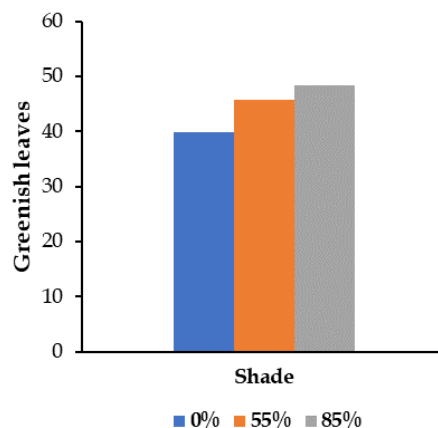


Figure 3. Bar chart of greenish leaves on sugar palm plants.

Based on the average results of greenery leaves on sugar palm seedlings, it can be concluded that the higher the percentage of shade, the denser the green color on the leaves. Shade treatment can affect chlorophyll content because the amount of light absorbed by plants is lower (Wulandari et al., 2016). To adapt to low light intensity, plants will increase light absorption by increasing the chlorophyll content per unit area of the leaf so that the green color of the leaves will be more concentrated (Sulistiyowati et al., 2019).

Fluctuations in the liquid organic fertilizer concentration and the combination of different shade percentages and liquid organic fertilizer concentrations did not show a significant difference in the green of the leaves based on the results of the F test, as the calculated F value was smaller than the F -table. Conditions in the

research environment can be affected by several factors, including temperature, humidity, light intensity, and precipitation. The light intensity distribution and the presence of precipitation may cause the light absorption of palm seeds to be different, so the shade treatment applied cannot tell the difference. Several factors determine the effectiveness of the use of liquid organic fertilizer, such as environmental factors, method of application, concentration, dosage, plant species, and the source of the organic matter used as a basis for producing liquid organic fertilizer, which also can fertilize. The treatment applied is no different (Haupt et al., 2021).

Conclusion

Treating different types of shadows yielded insignificant results for several variables, including total leaf area and chlorophyll, at both 0.55% and 85% shadows. Variations in liquid organic fertilizer concentration and combinations of different treatments in shade fraction and liquid organic fertilizer concentration also had no effect on any of the observed variables.

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Ruminta · Wicaksono FY · Nurinnida A · Wahyudin A

Identification of extreme weather and their correlation on soybean production in Garut regency

Abstract. The phenomenon of extreme weather events as a result of the impact of climate change can cause threats to agricultural systems, including soybean (*Glycine max* L.). Soybean is the main source of vegetable protein, which is sensitive and vulnerable to climate change. Therefore, research has been carried out through the identification analysis of changes in extreme weather events and analyzed for their correlation with soybean crops in Garut Regency to determine the effect of extreme weather elements on soybean production. The method used in this research is descriptive quantitative, using trend analysis on extreme weather with data on extreme weather elements such as maximum rainfall, maximum temperature, minimum temperature, wet spell, dry spell, the largest wind speed, and trend analysis on soybean production and productivity. Data for the research were obtained from BUTPAAG LAPAN Garut Regency, Garut Regency Agriculture Office, and other related sources. The correlation analysis used is the Pearson correlation with a significance level of 5%. The results showed that climate change impacts extreme weather changes in the Garut Regency area, with increasing extreme weather trends. However, extreme weather changes were not significantly correlated with soybean production. In this research, only the maximum rainfall and the largest wind speed were significantly correlated with soybean productivity.

Keywords: Correlation analysis · Extreme weather · Garut regency · Soybean production · Trend analysis

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Ruminta^{1*} · Wicaksono FY¹ · Nurinnida A² · Wahyudin A¹

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, Indonesia

² Study Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, Indonesia

*Correspondence: ruminta@unpad.ac.id.

Introduction

Climate change that occurs in Indonesia can impact changes in rainfall patterns, increasing temperatures and sea levels rise, and increasing the frequency and intensity of extreme weather events because climate change disrupts the global climate system and then causes an increase in the intensity and frequency of extreme weather events (Subagiyo, 2021; Stott, 2016; Cruz & Krausmann, 2013).

Extreme weather is an atmospheric change in a fairly short time at certain locations outside normal conditions that can cause hydrometeorological disasters such as drought, heavy rain, strong winds, and extreme temperatures (Surmaini & Faqih, 2016). The phenomenon of extreme weather as a result of the impact of climate change can cause threats to agricultural systems, including soybean crops (*Glycine max* L.). Soybean, as one of the food crops that is the main source of vegetable protein, is sensitive and vulnerable to climate change because it influences the decline in soybean production (Ruminta et al., 2020).

Along with the increasing population in Indonesia, the demand for soybeans also increases yearly. However, in recent decades soybean production has fluctuated. However, it tends to decline so that soybean production in Indonesia can only meet about 40% of domestic consumption and the remaining 60% is covered by imports (Carolina et al., 2016).

Soybean production, which tends to decline every year so that it has to import from abroad, is caused by the decline in soil fertility and the conversion of agricultural land into non-agricultural land. In addition, the occurrence of global climate change that can lead to extreme weather events is thought to be a difficult factor to control (Aminah et al., 2017; Araj et al., 2018; Fodor et al., 2017).

Extreme weather events due to climate change can threaten Garut Regency as the second largest soybean producer in West Java Province, with West Java's contribution to national soybean production of around 10% (Ministry of National Development Planning of the Republic of Indonesia, 2016). Garut Regency has a tropical climate with high rainfall, many rainy days, a very fertile area

with very high agricultural production capacity, and potential commodities, including soybean crops.

Identifying the number of extreme weather indicators such as maximum rainfall, maximum temperature, minimum temperature, consecutive rainy days (wet spell), days without consecutive rain (dry spell), and the largest wind speed that occurred in Garut Regency from 1982 to 2018 is important, given the fluctuations in soybean production in that year in Garut Regency because soybeans are a crop that is sensitive and vulnerable to climate change. Then also analyse the level of closeness (correlation) between the occurrence of extreme weather and changes in soybean production to help anticipate and mitigate disasters caused by extreme weather changes, such as droughts and floods, that can harm the agricultural sector, especially in soybean crops.

Materials and Methods

The research was conducted from April to August 2022. This research uses a quantitative descriptive method by using historical data in the form of extreme weather data including maximum rainfall, maximum temperature, minimum temperature, wet spell, dry spell, and the largest wind speed in 1982-2018 obtained from Balai Uji Teknologi dan Pengamatan Antariksa dan Atmosfer Garut, Lembaga Penerbangan dan Antariksa Nasional (BUTPAAG LAPAN) Garut Regency, as well as data on soybean crops including harvest area, production, and productivity obtained from the Garut Regency Agriculture Office. The climate data was divided into two periods (1982 to 2000 and 2001 to 2018) with almost the same amount of data to see climate change in both periods

The research data were analysed using trend and correlation analysis:

- 1) Trend line analysis (regression) using the following equation.

$$Y = b_0 + b_1X$$

$$b_0 = \frac{(\sum_{i=1}^n y_i)}{n}$$

$$b_1 = \frac{\sum_{i=1}^n (x_i y_i)}{\sum_{i=1}^n (x_i)^2}$$

where: Y = trend value of extreme weather data (maximum rainfall, maximum temperature, minimum temperature, wet spell, dry spell, or

largest wind speed) or soybean crop data (harvest area, production, or productivity); b_0 = constant value, which is the value of Y when the value of X = 0; b_1 = value of the slope of the line, which is the additional value of Y, if X increases by one unit; and X = year period.

- 2) Analyze the degree of closeness (correlation) between the occurrence of extreme weather changes and changes in soybean production using the following formula.

$$r = \frac{\sum_{i=1}^n x_i y_i - \frac{1}{n} (\sum_{i=1}^n x_i) (\sum_{i=1}^n y_i)}{\sqrt{(\sum_{i=1}^n x_i^2 - \frac{1}{n} (\sum_{i=1}^n x_i)^2) (\sum_{i=1}^n y_i^2 - \frac{1}{n} (\sum_{i=1}^n y_i)^2)}}$$

where: r = correlation coefficient; x_i = maximum rainfall data, maximum temperature, minimum temperature, wet spell, dry spell, greatest wind speed; y_i = data on harvest area, production, soybean productivity.

Data processing and analysis in this study used Microsoft excel and Minitab software version 19. The interpretation of the analysed data will be presented in the form of tables and charts.

Results and Discussion

Analysis of Extreme Weather Changes in Garut Regency. Based on the results of statistical test analysis for the last 37 years (1982-2018) the Garut Regency area has experienced extreme weather changes, which can be seen in Table 1 below.

Table 1 above shows that Garut Regency in period 1 (1982-2000) and period 2 (2001-2018) experienced an increase in maximum rainfall of 84.7 mm. The increase in maximum rainfall occurs due to the increase in temperature on earth, which causes an increase in evaporation events and the volume of water in cloud formation so that rain with higher intensity occurs (Puspitasari et al., 2016). The maximum average temperature and minimum average temperature also increased by 0.2°C and 0.1°C. By the research of Stocker et al. (2007) and Yang et al. (2021) the climate will continue to warm or increase in temperature over a certain period due to the emission of gases and carbon dioxide that will remain in the atmosphere. The average wet spells decreased by one day, inversely proportional to the increase in the average maximum rainfall, indicating that in Garut Regency, rainfall intensity is increasing, but the rainfall time is getting shorter. The average dry spells increased by four days, indicating an increasingly dry climate in the region. The average largest wind speed decreased by 14.9 km/h, although it decreased. However, the largest wind speed was included in the extreme category in both periods because the average wind speed, according to the operational standards of the BMKG, is between 5-30 km/h. The change analysis aligns with the following extreme weather elements trend analysis.

The Trend Analysis of Maximum Rainfall.

It can be seen in Figure 1 from the Y formula that a gradient of 6.82 mm is obtained, which means that the average annual maximum rainfall of Garut Regency has an upward trend of 6.82 mm each year.

Table 1. Extreme Weather Changes in Garut Regency on the period 1982-2000 and 2001-2018.

Climate Indicators	Extreme Weather Changes		Magnitude of Extreme Weather Change
	Period 1982-2000	Period 2001-2018	
Average Maximum Rainfall (mm)	153.9	238.6	84.7 mm
Maximum Average Temperature (°C)	28	28.2	0.2 °C
Minimum Average Temperature (°C)	24.8	24.9	0.1 °C
Average of Wet Spell	11	10	-1
Average of Dry Spell	23	27	4
Average Largest Wind Speed (km/hour)	52.5	37.6	-14.9 km/hour

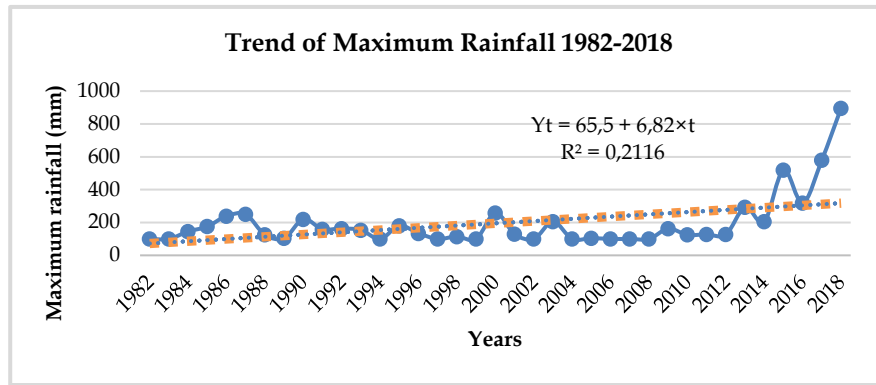
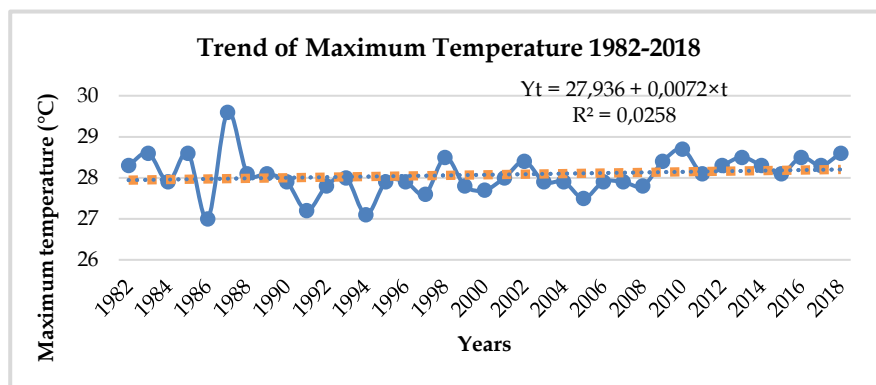
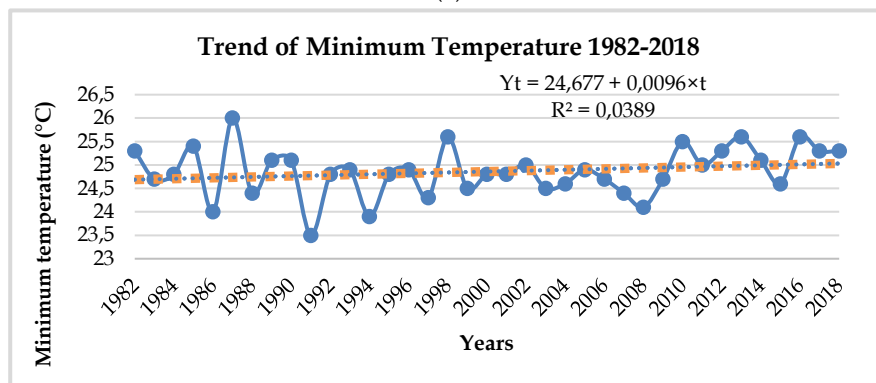


Figure 1. The trend of maximum rainfall in Garut Regency 1982-2018.



(a)



(b)

Figure 2. The trend of maximum temperature (a) and minimum temperature (b) in Garut Regency 1982-2018.

The Trend Analysis of Wet Spell and Dry Spell. It can be seen in Figure 3 (a) that the wet spell has a decreasing trend every year by 0.092

days. While the dry spell in Figure 3 (b) has a trend that continues to increase every year by 0.123 days.

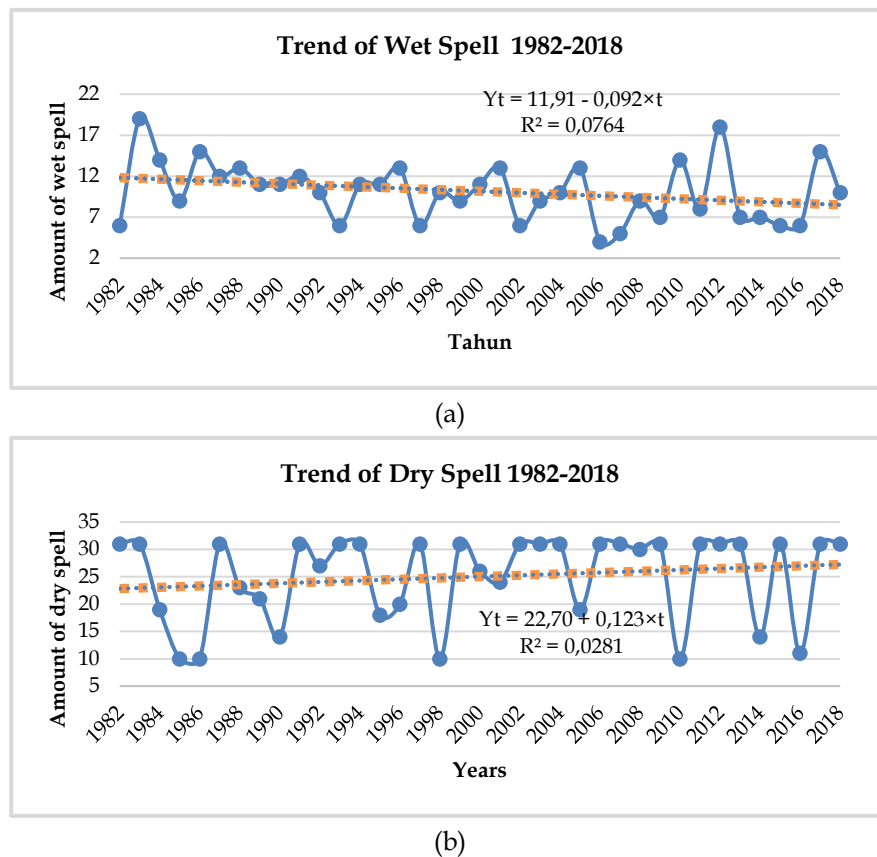


Figure 3. The trend of wet spell (a) and dry spell (b) in Garut Regency 1982-2018.

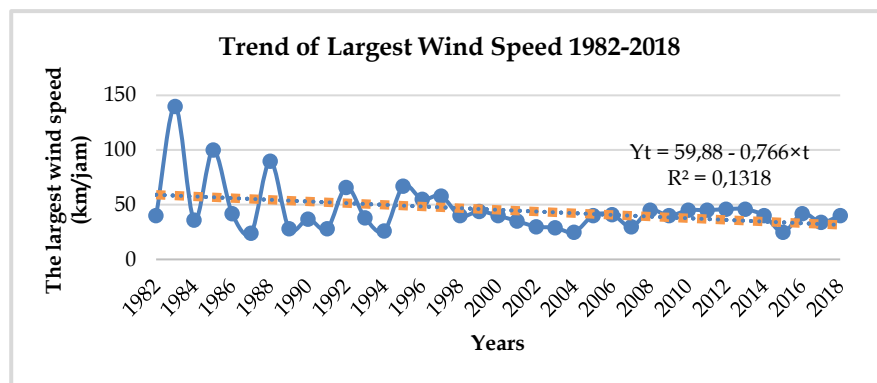


Figure 4. The trend of the greatest wind speed in Garut Regency in 1982-2018.

The Trend Analysis of Maximum Temperature and Minimum Temperature. It can be seen in Figure 2 (a) that the maximum air temperature in Garut Regency has an increasing trend of 0.007°C each year, and the minimum air temperature in Figure 2 (b) is 0.009°C each year.

The Trend Analysis of the Largest Wind Speed. It can be seen in Figure 4 that the most significant wind speed for 37 years in Garut

Regency has a downward trend of 0.766 km/h each year.

Analysis of Changes on Soybean Production and Productivity in Garut Regency. Based on the analysis results, which are divided into two periods, period 1 in 1982-2000 and period 2 in 2001-2018 in Garut Regency, it has experienced fluctuating changes which can be seen in Table 2 below.

Table 2. Changes in production, harvest area, and productivity of soybean in Garut Regency in the periods of 1982-2000 and 2001-2018.

Changes	Period 1982-2000	Period 2001-2018	Magnitude of Change
Production (tons)	32324.1	14522.8	-17801.3
Harvest area (ha)	25074.9	9406.6	-15668.3
Productivity (tons/ha)	1.231	1.526	0.295

Table 3. Correlation of extreme weather to soybean production in Garut Regency

Correlation	Production	Harvest area	Productivity
Maximum Rainfall	-0.086	-0.157	0.360 *
Maksimum Temperature	-0.293	-0.203	0.116
Minimum Temperature	-0.167	-0.136	0.188
Wet Spell	0.138	0.156	-0.212
Dry Spell	-0.168	-0.108	0.106
Largest Wind Speed	0.034	0.074	-0.362 *

Notes: (*) Significant

Table 2 above shows that soybean production decreased by 17,801.3 tons in 2 periods due to a decrease in soybean harvest areas during the two periods of 15,668.3 ha. The decline in the harvest area in the two periods was due to the conversion of agricultural land into non-agricultural land and the number of farmers who did not want to cultivate soybeans in Garut Regency because soybean farming was considered less profitable than other crops caused by inadequate soybean prices (Harsono, 2008). This is inversely proportional to the increase in productivity in the two periods of 0.295 tons/ha. The increase in soybean productivity in Garut Regency can cause some farmers there have begun to apply Integrated Crop Management (ICM) cultivation technology, such as most farmers always use the superior Anjasmoro variety, farmers also fertilise NPK according to the recommended dose, and weed control carried out by farmers has been carried out optimally.

Correlation of extreme weather changes to soybean production. The correlation technique used in this research is Pearson correlation, with a significance level of 5%. Correlation measures the strength and direction of the linear relationship between two variables: extreme weather and soybean crop. The results of the correlation analysis can be seen in Table 3 below.

Table 3 shows that only the extreme weather elements of maximum rainfall and largest wind speed are significantly correlated

with soybean crop productivity in Garut Regency. This is because some farmers in Garut Regency, although they have limited land area due to land conversion, have drainage channels on irrigated land that are good enough. So even if the rain intensity increases, it will not interfere with the growth of soybean plants, which causes their productivity to remain good. Supported by the research of Perdinan & Santikayasa (2006), Yang et al. (2020), and Kulig & Kopyra (2023) that the decreasing rainfall causes a decrease in soybean productivity in Bandung Regency by almost 50%, meaning that in Garut

In the district itself, maximum rainfall does not cause problems for soybean productivity as long as the drainage conditions on the land are good enough. While the decreased wind speed will minimise the risk of flower fall so that many pods are formed, which can increase soybean productivity because wind speed can affect the pollination process and determine the number of pods that will be formed (Kinasih et al., 2015; Zhang et al., 2017; Brittain et al., 2013).

None of the extreme weather elements in this research was significantly correlated with the decline in soybean crop production, it happened because the decline in soybean crop production in Garut Regency was mostly caused by the conversion of agricultural land, as happened in 2010 when rice fields decreased by 3 ha from 2009 and non-field land decreased by 649 ha compared to 2010 (Garut Agricultural Department, 2010). In addition, the decline in soybean production in Garut Regency is also caused by the low interest of farmers in cultivating soybean crops because

soybeans are only considered intercrops and are considered less profitable because the production costs are not comparable to the selling price when compared to other food crops such as rice and corn. Another influence is the effect of extreme weather such as flood or drought which increases the cost of soybean production.

Conclusion

There have been changes in extreme weather events due to the impact of climate change in Garut Regency with an increasing trend in maximum rainfall, maximum temperature, minimum temperature, and the number of days without rain in a row (dry spell) in two periods between 1982-2000 and 2001-2018, while extreme weather elements that have experienced a decreasing trend are wet spell and the largest wind speed. However, the occurrence of extreme weather in Garut Regency is not significantly correlated with changes in soybean crop production.

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Suherman C · Nurliawati SD · Ariyanti M · Anjarsari IRD · Soleh MA

Effectiveness of arbuscular mycorrhizal fungi in increasing growth and yield of maize overlaid on oil palm aged 4 years

Abstract. The intercropping system in oil palm plantations is an effort to optimize land, especially at the immature stages (IS), which have a large open space between the trees, so it can be used for cultivating annual crops such as maize. Oil palm trees are generally planted on marginal lands, such as Inceptisol, which generally lacks in phosphor (P). These problems can be reduced by applying arbuscular mycorrhizal fungi (AMF) to elevate P. This experiment was to determine the dosage and effectiveness of AMF that can improve the growth and yield of maize intercropped with a 4-year-old oil palm. The experiment was conducted at the Ciparanje Experimental Station, Faculty of Agriculture, Universitas Padjadjaran, from February to May 2022. The experiment used a randomized block design (RBD) with six treatments and was repeated four times. The treatment consisted of giving AMF doses, which included: without AMF, 2 g AMF/plant, 4 g AMF/plant, 6 g AMF/plant, 8 g AMF/plant, 10 g AMF/plant. The results showed that the application of AMF can increase growth and better yield maize. A dosage of 10 g AMF/plant is the best treatment, increasing plant height, cob length, cob diameter, dry shelled weight, and 100 seed weight, each 3, 04%, 5.5%, 8.1%, 50.21%, and 8.42% compared to no AMF.

Keywords: Arbuscular mycorrhizal fungi · Intercropping · Maize · Oil palm

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Suherman C^{1*} · Nurliawati SD² · Ariyanti M¹ · Anjarsari IRD¹ · Soleh MA¹

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang Km. 21, Sumedang 45363, Indonesia

² Undergraduate Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang Km. 21, Sumedang 45363, Indonesia

*Correspondence: cucu.suherman@unpad.ac.id

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is an important plantation crop in the agricultural sector. Oil palm trees have a good prospect, due to the highest oil production among other vegetable oil-producing plants (Agung et al., 2019).

The area of oil palm plantations in Indonesia continues to increase yearly. Based on the Ministry of Agriculture data, in 2021, the area of palm oil plantations reached 15.08 million ha, with the largest portion held by private large plantations (PLP). The proportion of oil palm plantation by land ownership amounted to 8.42 million ha (55.8%) of PLP, followed by smallholder plantations (SP) of 6.08 million ha (40.34%), and national plantations (NP) of 579.6 thousand ha (3.84%). In total, the oil palm area is 15.08 million Ha, consisting of 12,593,035 Ha (83.50%) Producing Plants (PP), 450,585 Ha (2.99%) are damaged plantations (DP), and 2,037,401 Ha (13.51%) are Immature Plants (IP) (Statistics Indonesia, 2021).

In Oil palm plantations, the trees are generally planted in an equilateral triangle pattern, with a spacing of 9 m x 9 m x 9 m with a population of 143 trees/ha. This wide spacing causes there is large open space among the trees at IS. This open space is potentially growing by many kinds of weeds leading to reduce soil fertility and harmful oil palm trees. To minimize this condition, the growing annual crop such as maize in the intercropping system can be an alternative in the oil palm plantation. Maize is the main food commodity after rice which has a strategic role in agricultural and economic development such as providing food and industrial raw materials. In Indonesia, the demand for maize always increases yearly (Ministry of Agriculture, 2021).

Good maintenance, such as fertilizer addition, can achieve high productivity of maize. One of the problems in oil palm plantations is that they are generally planted on marginal soils, such as inceptisols, which have limited chemical fertility, especially element P. Therefore, the way to increase the productivity of maize plants can be done by applying arbuscular mycorrhizal fungi (AMF) biofertilizers because they are capable of symbiosis with 90% of plant species (Smith & Read, 2008).

Arbuscular mycorrhizal fungi are inoculants made from active living organisms that facilitate the availability of nutrients, especially P, in the soil for plants (Husna et al., 2015). Utilization of this AMF is one of the alternatives in increasing soil fertility and supporting sustainable agriculture because it can reduce the use of fertilizers and inorganic materials to produce healthier maize feed. This study was to determine the dose of AMF that can improve the best growth and yield of maize intercropped with a 4-year-old oil palm

Materials and Methods

The experiment was carried out at the Ciparanje Experimental Garden, Faculty of Agriculture, Padjadjaran University, Jatinangor, an altitude of ± 780 m above sea level (asl). Inceptisol soil order, climate type C based on the classification of Schmidt and Ferguson (1951). The trial starts from February to May 2022.

The planting materials used were hybrid maize of the Pertiwi variety 3 and oil palm of the SEU Supreme variety, 48 months old. The other ingredients are NPK fertilizer, chicken manure, and consortium AMF inoculant, which consists of 4 species, namely *Gigaspora* sp., *Glomus* sp., *Entropospora* sp., and *Acaulospora* sp.

The design used was a randomized block design (RBD), consisting of six treatments and repeated four times so that there were 24 experimental units. The treatment plan consists of:

- A = without AMF
- B = 2 g AMF/plant
- C = 4 g AMF/plant
- D = 6 g AMF/plant
- E = 8 g AMF/plant
- F = 10 g AMF/plant

Observational data were tested by analysis of variance (Anova), if they were significantly different, the test was continued with Duncan's multiple range test at 95% confidence level (Gomez and Gomez, 1995)

The plant distance between maize and oil palm was 1.5 m. At the oil palms row there was maize trial plot of 4.5 m x 4.5 m The distance between maize plants was 75 cm x 20 cm and Corn planting is done between rows of oil

palms. Land preparation begins with clearing weeds and loosening the soil, then making beds.

Maintenance for maize plants includes fertilizing, hilling, weed control, watering, and pest and disease control. Basic fertilizer is given as chicken manure, as much as 15 tons/ha or 24 kg for one plot of maize plants. Further fertilization was carried out in the form of 300 kg/ha or 480 g/plot of urea, 100 kg/ha or 160 g/plot of SP-36, and 100 kg/ha of KCl or 160 g/plot. SP-36 and KCl fertilizers were given at 2 WAP, while urea fertilizer was given twice, namely at 2 WAP and 7 WAP.

The application of AMF was carried out once according to the treatment dose, carried out shortly before planting maize by mixing AMF in the planting hole, then planting maize seeds and covering them again with soil. Harvesting of maize plants was done when the maize cobs are ripe with the characteristics of yellowing leaves, yellowish husks, and brown hair on cobs. Variables observed included degree of root infection, index of chlorophyll content, stomatal conductance, plant height, stem diameter, leaf area, and yield components of maize plants (cob length, cob diameter, weight of 100 seeds, and weight of shelled seeds per plant).

Results and Discussion

Percentage of root infection. Table 1 shows that the treatment at a dose of 10 g AMF/plant resulted in a significantly different percentage response to root infection than the other treatments, but not significantly different from treatment E (8 g AMF/plant).

Table 2. Effect of AMF dosage on the chlorophyll content index of maize plant leaves among oil palm plantations.

Treatment	Chlorophyll content index (cci)			
	4 WAP	6 WAP	8 WAP	10 WAP
A (No AMF)	24.27 a	28.32 a	36.36 a	44.94 a
B (2 g AMF/Plant)	24.79 a	29.07 a	38.36 b	48.19 b
C (4 g AMF/Plant)	24.68 a	28.05 a	38.33 b	48.16 b
D (6 g AMF/Plant)	26.77 b	30.25 a	39.16 bc	47.97 b
E (8 g AMF/Plant)	26.78 b	29.15 a	39.09 bc	48.31 b
F (10 g AMF/Plant)	27.41 b	30.74 a	39.61 c	48.50 b

Note: WAP-weeks after planting. The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

The data shows that there is a tendency for the higher the application of AMF to maize plants, the higher the percentage of infection that occurs in plant roots. This is in accordance with the research of Jamilah et al. (2016); the higher the AMF inoculated in plants, the higher the percentage of AMF colonization on the roots.

Table 1. Effect of AMF dosage on the degree of root infection of maize plants among oil palm plantations age 4 years.

Perlakuan	Degree of Root Infection (%)
A (No AMF)	51.67 a
B (2 g AMF/Plant)	61.67 ab
C (4 g AMF/Plant)	60.00 a
D (6 g AMF/Plant)	63.34 ab
E (8 g AMF/Plant)	76.67 bc
F (10 g AMF/Plant)	79.17 c

Note: The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Mycorrhizal plant roots will be able to increase the capacity to absorb nutrients and water so that the absorption of nutrients needed by plants can run better. This situation is supported by a suitable growing environment for the development and activities of AMF (Suherman & Ridho, 2014).

Maize chlorophyll content index. Table 2 shows that, at the 8th and 10th WAP observations, all treatments with AMF doses produced a larger and significantly different chlorophyll content index than those without AMF.

Chlorophyll plays a role in capturing sunlight in the process of photosynthesis (Agustamia et al., 2016). A more efficient chlorophyll content in maize plants was found at 10 WAP observations. treatment of 2 g of AMF/plant produced the same chlorophyll content index. It was not significantly different from the result of higher AMF application, but significantly different from no AMF application. Providing more AMF besides being able to increase the absorption of nutrient P, other physiological aspects that are affected by AMF include water uptake (Davies et al., 1996), nitrogen fixation and resistance to root disease (Linderman, 1996), nitrogen element, plays an important role as a synthetic material chlorophyll, proteins, and amino acids.

Maize plant stomata conductance. Table 3 shows that, for each observation time, consistent doses of 10 g AMF/plant resulted in significantly different stomatal conductance than the treatment without AMF.

Stomata conductance will determine the yield, especially on the quality of maize seeds. Plants that have greater stomatal conductance have the potential to have high production (Sabagh et al., 2017). It is suspected that symbiotic mycorrhizae in plant roots will help plants more optimally absorb water molecules in the soil. This is in line with the opinion of Nyland (1996) who states that plants will grow well if the soil contains AMF because, through symbiosis, plants can absorb more water and nutrients.

According to Lestari (2006), plants colonized by AMF colonizes will obtain sufficient nutrients from the soil, especially the K element already available in the soil. K is an

essential nutrient in maintaining turgor pressure and regulating stomatal openings to improve plant growth. Stomata conductance is affected by carbon dioxide concentration, temperature, humidity, wind, light, and rainfall.

Plant height. Table 4 shows that, at the 4th week after planting (WAP), the application of AMF did not produce a significantly different plant height response but starting from the 6th, 8th, and 10th weeks of WAP observation, the application of AMF produced a different plant height response real.

Plant height at 8 and 10 WAP showed that all treatments with AMF produced a response to plant height that was higher and significantly different compared to the response to no AMF treatment. Data Table 4, shows that, a dose of 2 g of AMF/plant increased plant height by 2.6%, compared to no AMF, and was not significantly different from the height of plants given a higher AMF dose. This is in line with the effect of AMF dose on chlorophyll content index (Table 2.), that the application of 2 g AMF/plant, produced the same chlorophyll content index response as at a dose of 4-10 g AMF/plant. It is suspected that at this dosage, the presence of AMF is quite optimal, to support the availability of nutrients that enable good plant growth. One of the mechanisms by which AMF works in supporting nutrient availability is by releasing the phosphatase enzyme, which functions to break down unavailable P, to become available. The availability of elements that are difficult for plants to absorb becomes available to plants, will spur growth in plants (Zakiah and Fika, 2018). Application of 10 g of AMF/plant increased plant height by 3.04% compared to no AMF treatment.

Table 3. Effect of AMF dosage on stomatal conductance of maize plants among oil palm plantations.

Treatment	Stomatal Conductance (mmol H ₂ O/m ² s)			
	4 WAP	6 WAP	8 WAP	10 WAP
A (No AMF)	193.50 a	146.91 a	169.05 a	168.48 a
B (2 g AMF/Plant)	246.55 a	172.21 b	194.91 a	180.93 ab
C (4 g AMF/Plant)	251.71 a	168.56 b	183.85 a	189.72 ab
D (6 g AMF/Plant)	216.94 a	183.30 b	246.14 b	188.38 ab
E (8 g AMF/Plant)	264.16 ab	184.41 b	230.77 b	192.58 ab
F (10 g AMF/Plant)	337.17 b	186.39 b	260.94 b	199.66 b

Note: WAP-weeks after planting. The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Table 4. Effect of AMF dosage on maize plant height among oil palm plantations.

Treatment	Maize Plant Height (cm)			
	4 WAP	6 WAP	8 WAP	10 WAP
A (No AMF)	96.46 a	160.06 a	220.75 a	257.56 a
B (2 g AMF/Plant)	98.30 a	166.94 ab	230.81 b	264.16 b
C (4 g AMF/Plant)	98.56 a	168.31 ab	231.44 b	264.50 b
D (6 g AMF/Plant)	99.21 a	169.38 ab	230.38 b	264.38 b
E (8 g AMF/Plant)	98.40 a	168.59 ab	231.00 b	264.90 b
F (10 g AMF/Plant)	101.75 a	174.69 b	237.13 b	265.39 b

Note: WAP-weeks after planting. The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence

Table 5. Effect of AMF dosage on stalk diameter of maize plants among oil palm plantations.

Treatment	Stalk Diameter (cm)			
	4 WAP	6 WAP	8 WAP	10 WAP
A (No AMF)	1.70 a	2.51 a	2.64 a	2.76 a
B (2 g AMF/Plant)	1.71 a	2.54 a	2.66 a	2.77 a
C (4 g AMF/Plant)	1.73 a	2.51 a	2.65 a	2.80 a
D (6 g AMF/Plant)	1.72 a	2.51 a	2.62 a	2.80 a
E (8 g AMF/Plant)	1.72 a	2.51 a	2.66 a	2.81 a
F (10 g AMF/Plant)	1.72 a	2.57 a	2.67 a	2.83 a

Note: WAP-weeks after planting. The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Stalk diameter. Table 5 shows that all doses of AMF did not produce a significantly different maize stalk diameter response. Nevertheless, there is a tendency, an increase in the provision of AMF, numerically tends to increase the stem diameter. At 10 WAP observations, the application of AMF increased the diameter of the maize stalks by up to 2.5% compared to no AMF treatment.

The growth of maize stalk diameter is influenced by two factors, namely genetic factors and environmental factors such as soil fertility

Leaf area. Table 6 shows that the application of AMF doses did not produce significantly different leaf area responses in maize plants. This is presumably because the leaf area has increased significantly due to the low intensity of sunlight. According to Buntoro et al, (2014), the leaf area is affected by the intensity of sunlight. The low intensity of sunlight causes the thickness of the leaves to become thinner, but the leaf area increases significantly. Leaf area is closely related to the amount of photosynthesis produced by plants from photosynthesis. The greater the photosynthate produced by the plant, the greater the photosynthate translocated to the plant parts.

Maize cob length and diameter. Table 7 shows that the application of AMF doses resulted in significantly different responses to the length

and diameter of the maize cobs. Treatment at a dose of 10 g AMF/plant gave greater and significantly different cob length and diameter compared to cob length and diameter that were not treated with AMF. The calculation results show that giving 10 g of AMF/plant increases cob length by 5.5%, compared to not giving AMF. While the cob diameter increased by 8.1% compared to no AMF treatment. Roots infected with mycorrhizal fungi have a greater capacity to absorb nutrients than plants not infected with mycorrhizal fungi. (Sagala, 2013). In accordance with the opinion of Sintia (2011), providing balanced nutrients can increase maize yields in terms of cob quality.

Table 6. Effect of AMF dosage on maize leaf area among oil palm plantations.

Treatment	Leaf Area (cm ²)
A (No AMF)	711.68 a
B (2 g AMF/Plant)	713.43 a
C (4 g AMF/Plant)	705.43 a
D (6 g AMF/Plant)	714.41 a
E (8 g AMF/Plant)	723.02 a
F (10 g AMF/Plant)	728.45 a

Note: The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Table 7. Effect of AMF dosage on maize cob length and diameter among oil palm plantations.

Treatment	Cob Length (cm)	Cob Diameter (cm)
A (No AMF)	19.82 a	5.57 a
B (2 g AMF/Plant)	20.19 ab	5.68 ab
C (4 g AMF/Plant)	20.13 ab	5.64 a
D (6 g AMF/Plant)	20.13 ab	5.69 ab
E (8 g AMF/Plant)	20.50 ab	5.74 ab
F (10 g AMF/Plant)	20.91 b	6.02 b

Note: The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Based on the cob diameter data, mycorrhizal doses influence the formation of maize cob diameter. This is because of mycorrhiza can increase macronutrients and some microelements. According to Budiman (2004), element P is an element needed in large quantities in the formation of maize cobs. Low P conditions in the soil will increase the role of AMF in helping to release total P to become available P because AMF produces organic acids which can release unavailable P to become available P (Syamsiyah et al., 2014).

Dry-shelled weight per plant and 100 maize kernels. Table 8 shows that, in general, the application of AMF increased the weight of dry-shelled seeds planted between 36.06-50.21%, compared to no AMF treatment. Treatment at a dose of 4 g AMF/plant resulted in higher dry shell weight/plant and was significantly different than without AMF, but not significantly different from the effect of other treatments. Giving AMF to maize plants can increase the absorption of plant nutrients so that it has a significant effect on maize seed production

This study's results align with Sukiman's study (2010), which showed an increase in the yield of dry-shelled maize with AMF application could provide better results than without AMF application. This is presumably because AMF-infected plants, through their external hyphae network, expand the penetration of root uptake so that the plants obtain an adequate supply of nutrients for growth and an increase in maize yields.

The application of AMF also significantly affected the weight of 100 seeds (Table 9). The table shows that the provision of AMF increased

the weight of 100 seeds between 2.00-8.42% compared to no AMF. Dosing 10 g AMF/plant produced a larger and significantly different weight of 100 seeds compared to no AMF treatment, but not significantly different from the other treatments.

Table 8. Effect of AMF dosage on dry seed weight among oil palm plantations

Treatment	Dry Seed Weight per plant (g)
A (No AMF)	89.19 a
B (2 g AMF/Plant)	125.96 ab
C (4 g AMF/Plant)	129.93 b
D (6 g AMF/Plant)	121.35 ab
E (8 g AMF/Plant)	133.12 b
F (10 g AMF/Plant)	133.97 b

Note: The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

Table 9. Effect of AMF dosage on the weight of 100 maize seeds between oil palm plantations

Treatment	Weight of 100 Maize Seeds (g)
A (No AMF)	21.27 a
B (2 g AMF/Plant)	21.94 ab
C (4 g AMF/Plant)	22.48 ab
D (6 g AMF/Plant)	21.69 ab
E (8 g AMF/Plant)	22.51 ab
F (10 g AMF/Plant)	23.06 b

Note: The mean value followed by the same letter in the same column is not significantly different according to Duncan's multiple range test at the 95% level of confidence.

The higher dried weight of seeds per plant was supported by the formation of larger seeds. This is indicated by the higher weight of 100 seeds. The weight of 100 dry-shelled maize kernels is largely determined by photosynthate accumulation during the seed filling phase. The presence of AMF in the roots of maize plants allows for increased absorption of water and nutrients, including P nutrients by plants, so that they can meet plant needs in the process of forming and filling seeds (Kuik, 2022).

Conclusion

1. The application of AMF effectively increase growth and yield of maize plants. Plants

treated with AMF have better results than plants without AMF.

2. Giving a dose of 10 g AMF/plant is the best treatment, increasing plant height, cob length, cob diameter, dry shelled weight, and 100 seed weight, respectively 3,04%, 5,5%, 8,1%, 50,21%, and 8,42% compared to no AMF.

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Khafid A · Nurchayati Y · Hastuti ED · Setiari N

Vitamin C and total soluble solid content of crystal guava at different storage duration and ripeness

Abstract. Crystal guava (*Psidium guajava* var. 'Crystal') fruit is in great demand because of its delicious taste and high nutritional content. Storage aims to prevent postharvest damage to the fruit. However, storage that is too long causes morphological damage and decreased nutrients. This study aims to determine the effect of storage duration, fruit ripeness stage and the interaction between both factors on vitamin C and total soluble solids (TSS) content of crystal guava, and determine which treatment can produce the highest vitamin C and TSS. Fruits harvested simultaneously with three levels of ripeness based on the skin color: unripe fruit is dark green, ripe is light green, very ripe is yellowish green. Samples selected based on the same weight range. Storage was carried out for 0, 5, and 10 days at $\pm 10^{\circ}\text{C}$. The study used a completely randomized design (CRD) with a 3x3 factorial pattern with two factors: storage duration and fruit ripeness level. Parameters observed were vitamin C, TSS, weight loss, diameter shrinkage, skin color and hardness. Data were analyzed using ANOVA and DMRT. Both treatments showed an interaction on vitamin C content. The best treatment was unripe fruit stored for ten days with 14.955 ppm of vitamin C. Both treatments did not show any interaction on TSS content. The best treatment was five days storage with TSS of 8.25 °Brix and very ripe fruit of 8.21 °Brix. Based on vitamin C, TSS content, and physical condition variables, the best guava fruit is unripe fruit stored for 10 days.

Keywords: Color · Physical · Postharvest · Quality · Softening

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Khafid A¹ · Nurchayati Y^{1*} · Hastuti ED¹ · Setiari N¹

¹ Departement of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof Soedarto, SH. Tembalang, Semarang City 50275, Indonesia

*Correspondence: yulita.yoko@gmail.com

Introduction

Crystal guava (*Psidium guajava* var. 'crystal') is a plant from the Myrtaceae family and is a cultivar of guava. Crystal guava is in great demand because it has a crunchy texture with few seeds and has many health benefits (Rosmalova, 2021), including helping to ward off free radicals, boosting the immune system, helping to maintain the health of heart, skin and digestive system (Sasmi et al., 2022).

People usually prefer to consume fruit with the best quality. Crystal guava fruit quality can be assessed from several aspects, such as physical condition, which includes fruit skin, hardness, color, and fruit size, as well as from the nutritional aspects such as total soluble solids (TSS), vitamin C and total acid (Kalsum et al., 2018; Kusumiyati et al., 2018; Romalasari et al., 2017).

Physical condition is one of the important criteria in determining fruit quality, because it can be seen directly. Fruits that have good physical condition are more attractive to consumers (Sembiring et al., 2020). Total soluble solids (TSS) can be used as fruit sweetness indicator because TSS component mostly are sugar compounds (Liu et al., 2010). This causes fruit that has a high TSS content to have a sweeter taste, so consumers prefer it. The content of vitamin C can also be used as fruit quality determiner because it is known to have health benefits when consumed, such as boost immune system (Carr & Maggini, 2017), as an antioxidant (Durán-Soria et al., 2021), and is important for skin health (Pullar et al., 2017). This is what causes fruit with a high vitamin C content to be considered good quality fruit.

One of the fruit quality determiner factors is its ripeness level. During the fruit ripening process, a series of physiological processes occur which cause changes in the biochemical content and structure of the fruit, such as pigments synthesis, starch breakdown, sugar, volatile compounds, and secondary metabolites synthesis (Pullar et al., 2017). Unripe fruits taste and texture are less delicious to consume, but the nutrients contained in them are still low compared to ripe fruits. So, the more ripe a fruit is, the better its quality (Maduwanthi & Marapana, 2017; Trong et al., 2019). However, fruit that is too ripe will also have decreased quality. Overripe fruit color will change, have a strong scent and the fruit will

become soft, making it easily damaged and get caught by disease (Pott et al., 2020).

Generally, fruits that been harvested by farmers are not sold immediately to consumers. This creates a time gap between the harvest and the consumption where fruits are being prone to be damaged. To prevent this, the fruit that has been harvested must be given a series of postharvest treatments. Postharvest treatments aims to minimize the level of damage to plant products after being harvested until they sold consumers (Mutiarawati, 2007). Storage is one of the postharvest treatments that aims to protect the fruit from damage (Sudjatha & Wisaniyasa, 2017). However, too long storage can also cause morphological damage to the fruit and reduce some of its nutritional value (Hong et al., 2012).

This study aims to determine the effect of storage duration, ripeness stage and their interaction on crystal guava fruit vitamin C and total soluble solids (TSS) content, and determine which treatment combination can produce the highest vitamin C and TSS.

Materials and Methods

This research was conducted in March-May 2022. Sampling was carried out at a crystal guava plantation in Kandri Village, Gunungpati District, Semarang City. Preparation and determination of vitamin C content was carried out at the Plant Structure and Function Biology Laboratory Department of Biology, Faculty of Science and Mathematics, Diponegoro University, and TSS content determination was carried out at the Food Engineering and Agricultural Products Engineering Laboratory, Faculty of Animal and Agricultural Science, Diponegoro University.

The materials used in this study included crystal guava fruit (*Psidium guajava* var. 'Crystal') which harvested from 6 years old tree, ascorbic acid and distilled water.

Research Design. The design used in this study was a 3x3 factorial complete randomized design (CRD). The first factor is the ripeness level of the crystal guava fruit, including unripe, ripe and very ripe fruit taking into account the color and the appearance of the fruit skin. The second factor was crystal guava's storage duration, including 0, 5, and 10 days in the refrigerator ($\pm 10^{\circ}\text{C}$). Total guava fruit used in this research are 45 fruits. Vitamin C test was subjected to nine

treatments with three replications each, so the total was 27 fruits. While TSS test was subjected to nine treatments with two replications each, so the total was 18 fruits.

Table 1. Research Design

Maturity	Storage Duration		
	S1	S2	S3
M1	M1S1	M1S2	M1S3
M2	M2S1	M2S2	M2S3
M3	M3S1	M3S2	M3S3

Note :

M1 : Unripe S1 : 0 day storage
M2 : Ripe S2 : 5 day storage
M3 : Very Ripe S3 : 10 day storage

Crystal Guava Fruit Harvest and Selection.

Crystal guava fruit is harvested simultaneously based on three levels of ripeness based the fruit skin color: unripe fruit is dark green, ripe fruit is light green and very ripe fruit is yellowish green. Crystal guava fruit then is weighed and selected based on the same weight range (200-250 gr). The fruits that were selected are also free of open or severe wounds.



Figure 1. Guava's Ripeness Level Based on Skin Color : (a) Unripe – Dark Green (b) Ripe – Light Green (c) Very Ripe – Yellowish Green

Storage. Guava Crystals that had been cleaned by wiping off dirt or other substances from fruit surface, wrapped in plastic wrap, and then stored in a plastic bag in the refrigerator at $\pm 10^{\circ}\text{C}$ for 0 days, 5 days and 10 days.

Making CO_2 Free Aquadest. Making ascorbic acid standart solution or to do extraction from fruit requires CO_2 -free distilled water. CO_2 -free distilled water is made by heating distilled water for 5-10 minutes. After it boils, the distilled water is then left to cool into room temperature (Fauzana et al., 2022)

Test of Vitamin C. Testing for Vitamin C content was carried out destructively using the spectrophotometric method based on research conducted by Herlina & Muzdalifa (2020) with some modifications. The steps taken are as follows:

1) Determination of the Maximum Wavelength of Ascorbic Acid

Ascorbic acid was used as a standard solution to determine vitamin C content using spectrophotometry method. An ascorbic acid solution with a concentration of 10 ppm was prepared, then the absorbance value was measured using a UV-Vis spectrophotometer with a series of wavelengths ranging from 260 to 270 nm (Dewi et al., 2018).

The absorbance value of the measurement results will form a curve. The curve obtained will show the wavelength that produces the highest absorbance value. This wavelength is used to determine the vitamin C content of the sample.

2) Creating a Calibration Curve

Serial of ascorbic acid dilutions were carried out with levels of 2, 4, 6, 8, 10 ppm. The ascorbic acid solution was measured for its absorbance value using the maximum wavelength that was previously obtained. A line equation graph is made where the x-axis is the concentration of ascorbic acid (ppm) and the y-axis is the absorbance value. The line obtained from the graph is a line equation with the formula $y = bx + a$.

3) Determination of Vitamin C Content

Crystal guava fruit mashed with a mortar. Sample weighed 0.25 g, then put into a test tube, then added 5 ml of distilled water and centrifuged at 3500 rpm for 15 minutes. The sample was then filtered using filter paper and poured into an Erlenmeyer and then added 12 ml of distilled water. The sample then measured its absorbance value with a spectrophotometer with the optimum wavelength that had been obtained previously. The concentration of vitamin C is obtained by putting the absorbance value of the sample into the linear equation $y = bx + a$ where y is the absorbance value, x is the concentration of vitamin C, a is a constant and b is the coefficient.

Test of TSS. TSS were measured destructively using a digital refractometer. Crystal guava fruit is crushed using food processor, then the extract is taken and dripped on the refractometer lens. The results will then appear automatically on the refractometer display. The units for the amount of TSS shown are units in $^{\circ}\text{Brix}$ (Ana et al., 2021).

Fruit's Weight Loss Calculation. Crystal Guava weighed using scale before and after storage, then the weight loss percentage was calculated using the formula below :

$$\text{Weight Loss} = \frac{W_1 - W_n}{W_1} \times 100\%$$

Note :

W₁ = Initial weight (gr)

W_n = Weight day-n storage (gr)

Fruit's Diameter Shrinkage Calculation.

Crystal Guava diameter was measured using caliper before and after storage, then the diameter loss percentage was calculated using the formula below :

$$\text{Diameter Shrinkage} = \frac{d_1 - d_n}{d_1} \times 100\%$$

Note :

d₁ = Initial fruit diameter (mm)

d_n = Fruit diameter day-n Storage (mm)

Fruit Physical Changes. Fruit Physical Changes were analyzed descriptively by observing fruit conditions before and after storage. Parameters observed included fruit color and hardness. Documentation is also carried out before and after the fruit is stored.

Data analysis. Quantitative data obtained from the study included vitamin C content, TSS, weight loss and diameter were analyzed using Two-Way ANOVA to see whether or not there was a significant effect between treatments. If there is, the analysis is carried on with DMRT (Duncan's Multiple Range Test) at a significance level of 95% to determine the difference between treatments. Qualitative data including fruit visual, changes in fruit skin color and fruit hardness were observed and presented descriptively accompanied by documentation in the form of photos from the observations.

Result and Discussion

Ascorbic acid was used as a standard solution to determine the levels of vitamin C contained in crystal guava fruit using the spectrophotometric method. To determine the wavelength to be used, it is necessary to make a maximum wavelength curve beforehand. The ascorbic acid solution absorbance value measured at a wavelength of 260 to 270 nm (Dewi et al., 2018). Based on the measurements that have been made, the highest absorbance value is 0.483 at a wavelength of 265

nm (Figure 1.). Therefore, the wavelength used to measure the absorbance of vitamin C in crystal guava fruit is 265 nm.

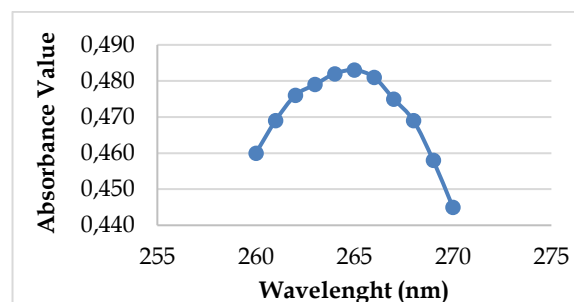


Figure 2. Maximum wavelength absorption curve for ascorbic acid.

Standard curve serves to determine the vitamin C content in a sample based on its absorbance value. The preparation of a standard curve was carried out by measuring the absorbance value of ascorbic acid solutions in stages with concentrations of 2, 4, 6, 8, and 10 ppm. Based on the test results, a standard curve is obtained with the line equation $y = 0.0799x - 0.0209$ (Figure 2.). The vitamin C content is determined by putting the absorbance value into the line equation that has been obtained, where the y variable is the absorbance value and the x variable is the vitamin C concentration.

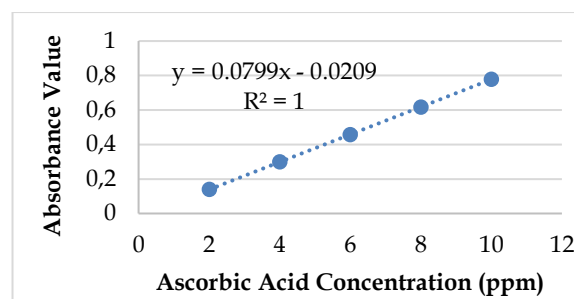


Figure 3. Ascorbic Acid Standard Curve

The results of ANOVA showed that the combination of duration of storage and ripeness level treatment had a significant effect on the vitamin C content of crystal guava fruit. The highest vitamin C content was found in the M1S3 treatment with 14.955 ppm. The lowest vitamin C content was found in the M1S1 treatment of 5.760 ppm (Table 2).

Unripe crystal guava fruit showed an increase in vitamin C content until the 10th day of storage (Table 2.). This is presumably because the fruit continues to produce vitamin C during storage. Kubo (2014) explained that storage in

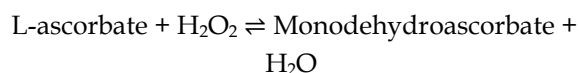
cold temperatures (0-15 °C) is known to reduce the rate of metabolism in fruit, but not all metabolic processes are stopped or suppressed to the same extent. Tsaniklidis et al. (2014) in their research showed that enzymes that play a role in the synthesis of ascorbic acid such as L-galactono 1,4 lactone dehydrogenase (GalLDH) and GDP-Mannose-3'5'-epimerase (GME) were still active in tomatoes stored at room temperature. 10° C

Table 2. Content of vitamin C (ppm) of crystal guava fruit at different treatments of storage duration and ripeness level.

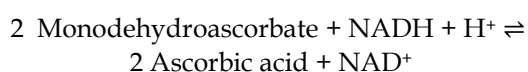
Treatment	Storage Duration			Mean
	S1	S2	S3	
M1	5.760 ⁱ	8.933 ^{gh}	14.955 ^{abc}	9.883
M2	7.183 ^{hi}	12.541 ^{cde}	9.995 ^{efg}	9.906
M3	9.882 ^{fg}	11.346 ^{def}	14.874 ^{bc}	12.034
Mean	7.608	10.940	13.274	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% ($\alpha=5\%$)

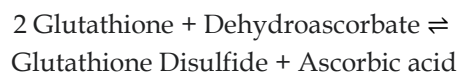
Cells experienced stress in the form of chilling injury due to low temperature storage in the range of 15 °C to 0 °C (Kubo, 2014). Plants produce high amounts of H₂O₂ which are Reactive Oxidative Species (ROS) as a response to cold stress (Slesak et al., 2007). ROS compounds can cause cell damage that leads to cell death (Smirnoff & Wheeler, 2000). Ascorbic acid has the main function as a reducing agent for H₂O₂ which is produced both in the process of photosynthesis and in stress (Tsaniklidis et al., 2014). Wang et al. (2017) stated that ascorbic acid acts as a cofactor for the enzyme ascorbate peroxidase (APx) which plays a role in reducing H₂O₂, producing monodehydroascorbate (MDHA) and H₂O. This process occurs in the cell wall.



The amount of ascorbic acid in fruit remains high in cold storage due to the recycle. Smirnoff (2018) explained that monodehydroascorbate originating from the oxidation process by the APx enzyme will be reduced by the monodehydroascorbate reductase (MDHAR) enzyme using NADH, producing ascorbic acid again.



There are conditions where MDHA can be broken down into ascorbic acid and dehydroascorbic (DHA). Ascorbic acid can also be produced from the reduction of DHA by the enzyme dehydroascorbate reductase (DHAR) with the help of glutathione which is an antioxidant compound.



The vitamin C content in unripe crystal guava fruit was lower at 0 and 5 days of storage because the fruit was not fully ripe yet. The content of vitamin C crystal guava fruit is higher when the fruit becomes more ripe. Kartika (2016) states that the highest vitamin C content occurs when the fruit becomes more ripe. This increase occurred due to the biosynthesis of vitamin C from glucose contained in the fruit. Fenech et al. (2019) explained that the most dominant biosynthesis of ascorbic acid in plants occurs through the Smirnoff-Wheeler (SW) synthesis pathway. Ascorbic acid in this pathway is synthesized from the sugar molecule D-glucose-6-phosphate. The sugar content in ripe fruit is higher than in unripe fruit (Dewi et al., 2017; Dolkar et al., 2017), so that ripe fruit can produce more vitamin C because there is more substrate available.

Crystal guava fruit that is very ripe also experiences a similar condition to unripe fruit. The vitamin C content of very ripe fruit also increases up to 10 days of storage. In addition, the vitamin C content of very ripe fruit was higher than other fruit at 0 and 5 days of storage. The vitamin C content of very ripe fruit on the 10th day of storage tends to be the same as that of unripe fruit, although it is lower.

The high content of vitamin C in very ripe fruit in each storage treatment is thought to be related to its ripeness level. Fruits can synthesize ascorbic acid in greater quantities due to the supply of additional substrates for synthesizing ascorbic acid, one of which is pectin. This pectin comes from the process of softening the cell walls of the fruit, where when the fruit becomes more ripe, hydrolysis of pectin compounds will occur in the cell walls (Sharma et al., 2015). This pectin will then be used as a substrate for the synthesis of ascorbic acid via the D-galacturonic pathway (Fenech et al., 2019). This is also reinforced by the condition of very ripe fruit, which has a softer

fruit hardness level than other ripeness levels (Table 8.).

Ripe crystal guava fruit vitamin C content increase at 5 days of storage, then decreased at 10 days of storage (Table 2.). This is presumably because vitamin C is used as an oxidative defense, while glucose, which is used as a substrate for synthesizing vitamin C, is diverted to the respiration process which increases in the climacteric phase of ripe fruit. The climacteric phase is a phase that occurs in several types of fruit where there will be a drastic increase in respiration rate (Saltveit, 2019). Low temperatures can reduce respiration rate, but not completely stopped (Bal, 2013; Luengwilai & Beckles, 2013).

Saltveit (2019) explained that carbohydrates will be broken down into glucose, then through the process of glycolysis, glucose is processed to produce other compounds, such as pyruvic acid, NADPH and ATP and glucose-6-phosphate. Glucose and Glucose-6-phosphate are substrates that act as materials for synthesizing vitamin C. In the process of respiration, these two compounds are used to produce energy. Glucose is used through the Krebs cycle process and oxidative carboxylation (glucose) to produce ATP and NADPH, while glucose-6-phosphate is used through the Pentose-Phosphate Shunt process or also called the phosphoglutanean pathway.

Based on the results of ANOVA, the combination of treatments between storage duration and ripeness level did not show any interaction with total soluble solids (TSS) content. However, each variable independently shows a significant influence. The best storage duration treatment was treatment P2, with an average TSS content of 8.25 °Brix and the lowest TSS content in treatments P1 and P3 were 7.23 and 7.01 °Brix, respectively. The level of fruit ripeness with the highest TSS content was at T3 of 8.21 °Brix and the lowest was at T1 of 6.95 °Brix and T2 of 7.33 °Brix (Table 3).

The total soluble solids (TSS) content of crystal guava increased in more ripe fruit. In the storage duration treatment, the TSS content of crystal guava also increased at 5 days of storage (Table 3.). The same thing was also reported by Dolkar et al. (2017) and Hong et al. (2012) where the TSS content of guava fruit increases in more ripe fruit and in longer storage. This is because during storage, hydrolysis of starch compounds occurs, changing them from insoluble to became soluble (Bishnoi et al., 2017; Dolkar et al., 2017).

Table 3. Tss content (°brix) of crystal guava fruit at different treatments of storage duration and ripeness level.

Ripeness	Storage Duration			
	S1	S2	S3	Mean
M1	7.05	7.10	6.70	6.95 ^b
M2	6.63	8.53	6.85	7.33 ^b
M3	8.03	9.13	7.48	8.21 ^a
Mean	7.23 ^a	8.25 ^p	7.01 ^a	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% ($\alpha=5\%$)

Sharma et al. (2015) stated that guava produces various enzymes that break down polysaccharide compounds, including the enzymes pectin galacturonase (PG), pectin methyl esterase (PME), cellulase and β -D-galactosidase. Payasi et al. (2009) explained that PG enzymes play a role in hydrolyzing glycosidic bonds in galacturonic acid which is a component of pectin. The PME enzyme works by removing the methyl group from galacturonic acid from pectin. Cellulase enzymes play a role in hydrolyzing β -1,4 glucan bonds in cellulose and xyloglucan. The β -D-galactosidase enzyme plays a role in removing galactosyl groups in pectin and xyloglucan. This is confirmed by Dolkar et al. (2017) research, which is the pectin content in guava decreased when it became more ripe along with increasing PME enzyme activity. This was supported by the finding of decrease in hardness during cold storage in guavas (Hong et al., 2012), tomatoes (Luengwilai & Beckles, 2013) and plums (Bal, 2013).

The TSS content of crystal guava decreased at 10 days of storage in all ripeness level (Table 3). The decrease in TSS occurred because of the sugar compounds in the fruit were used for various metabolic processes, such as for the biosynthesis of vitamin C (Fenech et al., 2019), fruit development and as a source of energy (Durán-Soria et al., 2021; Saltveit, 2019). This is also supported by the increase of crystal guava fruit vitamin C content during storage (Table 2), where the content of sugar compounds is partly intended to synthesize vitamin C as a defense mechanism from oxidative stress due to low temperatures.

The results of ANOVA showed that there was no interaction between the combination of ripeness level and storage duration treatments on crystal guava fruit weight loss. When viewed from each treatment individually, the ripeness

level did not have a significant effect on weight loss (Table 4), but there was a tendency for the highest fruit weight loss to occur in the T2 ripeness treatment, which was 0.004%. Storage duration treatment has a significant effect on fruit weight loss. Storage duration that causes the highest weight loss is the P3 of 0.005%.

Table 4. Weight loss (%) of crystal guava fruit at different treatments of storage duration and ripeness levels.

Ripeness	Storage Duration		
	S2	S3	Mean
M1	0.001	0.004	0.002 ^a
M2	0.001	0.007	0.004 ^a
M3	0.001	0.004	0.002 ^a
Mean	0.001 ^q	0.005 ^p	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% ($\alpha=5\%$)

Crystal guava fruit weight were loss during 5 and 10 days of storage (Table 4). Fruit weight loss occurs due to reduced water content in the fruit, due to the fact that the fruit stored after being harvested still undergoes a transpiration process. Transpiration is a process where water molecules from inside the fruit cells will come out into the environment. The longer the storage duration, the longer the transpiration process takes, so that the weight loss is also higher (Lawati et al., 2021; Kalsum et al., 2018). The rate of weight loss is influenced by several aspects, including environmental factors such as temperature and humidity (Kusumiyati et al., 2018).

There was a tendency for weight loss to be higher in ripe crystal guava fruit compared to unripe and very ripe fruit. This is because the ripe crystal guava fruit is in the climacteric phase, where the rate of cellular respiration is at its peak. Respiration is the process of breaking down sugar compounds stored in cells. Sugar compounds such as glucose are broken down into CO₂ and H₂O and produce energy that will be used in the development process (Saltveit, 2019). The breakdown of glucose compounds causes the biomass of crystalline guava to decrease. Kusumiyati et al. (2018) stated that the fruit that has been harvested continues to undergo metabolism, where the food reserves stored in the vacuoles will be used, while the fruit no longer gets a supply of nutrients from the tree because it has been picked.

The results of ANOVA showed that there was no interaction between storage duration and










fruit maturity level on the diameter shrinkage of crystal guava fruit. Likewise, each treatment independently did not have a significant effect. The highest weight loss was found in immature crystal guava fruit and fruit stored for 10 days (Table 5.)

Table 5. Diameter shrinkage (%) of crystal guava fruit at different treatment duration of storage and ripeness levels.

Ripeness	Storage Duration		
	S2	S3	Mean
M1	0.002	0.005	0.003 ^a
M2	0.001	0.004	0.002 ^a
M3	0.000	0.002	0.001 ^a
Mean	0.001 ^p	0.003 ^p	

Note: Numbers followed by the same letters are not significantly different in the DMRT test at a significance level of 95% ($\alpha=5\%$)

Table 6. Visual of Crystal Guava Fruit at Different Treatments of Storage Duration and Ripeness Levels

Ripeness	Storage Duration		
	S1	S2	S3
M1			
M2			
M3			

In Table 5., it can be seen that unripe fruit after being stored for 5 the diameter are shrunk by 0.002% from the initial diameter, after being stored for up to 10 days, the diameter shrinkage increased to 0.005%. Ripe fruit diameter shrunk by 0.001% from the initial diameter after being stored for 5 days, and increased to 0.004% after being stored for up to 10 days. Very ripe fruit when stored for 5 days did not experience a diameter shrinkage, but after being stored for 10 days the diameter shrunk by 0.002% from the initial diameter. The average shrinkage in diameter for 5 days of storage was 0.001% and for 10 days of storage was 0.003%. The reduction in fruit diameter is thought to be due to the transpiration process in the fruit. Transpiration

causes a reduction in the number of water molecules contained in cells because they move out into the environment (Lawati et al., 2021). This causes the cell volume to decrease, resulting in a reduced size of the crystal guava fruit.

Analysis of the physical condition of crystal guava fruit was carried out qualitatively. The physical conditions of the fruit are presented in tabular form including visual fruit (Table 6.), skin color (Table 7.) and fruit hardness (Table 8.). There are differences in the physical conditions of crystal guava fruit with different ripeness levels and after being stored at different duration.

Based on observations, unripe has a dark green skin color. After being stored for 5 and 10 days, the color of the unripe fruit skin changed to yellowish green. Ripe fruit has a bright green skin color, and turns yellowish green on storage for 5 and 10 days. Very ripe fruit has a yellowish green skin color, and turns greenish yellow after 5 days of storage, then becomes bright yellow after 10 days of storage. Color scale were created to help to understand more of the color difference between treatments (Table 7).

Table 7. Skin color number scale of crystal guava fruit at different treatments of storage duration and level of ripeness.

Ripeness	Storage Duration		
	S1	S2	S3
M1	1	3	3
M2	2	3	3
M3	3	4	5

Description: 1 = dark green; 2 = Bright green; 3 = Yellowish green; 4 = Greenish yellow; 5 = Bright yellow

The color change that occurs in the skin of the crystal guava is due to the process of changing the pigment in the fruit skin. This color change also indicates that the fruit is becoming more ripe. Choo (2018) stated that chlorophyll degradation causes the color of the fruit to change. Kapoor et al. (2022) added that ethylene produced during fruit ripening functions as a signaling compound, where ethylene signals specific transcription factors, activating chlorophyll catabolic genes (CCG) which play a role in degrading chlorophyll in fruit peels.

There are differences in the level of fruit hardness in fruit with different ripeness. The fruit also experienced changes in the level of hardness after being stored for different durations (Table 8). Unripe fruit has a hardness rating on the very

hard scale. After being stored for 5 and 10 days, the fruit becomes softer, the level of hardness becomes "hard". Ripe fruit has a hardness level on the "hard" scale, then after being stored for 10 days it becomes softer, the hardness level becomes "soft". Very ripe fruit has a hardness level on the "hard" scale, changing to "soft" after being stored for 5 days and becoming "very soft" after being stored for 10 days (Table 8).

Table 8. Hardness of crystal guava fruit at different treatments of storage duration and ripeness levels.

Ripeness	Storage Duration		
	S1	S2	S3
M1	++++	+++	+++
M2	+++	+++	++
M3	+++	++	+

Note : ++++ = Very Hard; +++ = Hard; ++ = Soft; + = Very soft

This change in hardness of the crystal guava fruit is due to the softening of the fruit cell walls during fruit ripening. Pectin is one of the dominant compounds in composing the middle lamella. During maturation, changes occur in the parenchyma cell wall, especially in the middle lamella. Pectin undergoes a change from insoluble to dissolved (Paniagua et al., 2014; Payasi et al., 2009). Several enzymes play a role in this process, including the pectin galacturonase (PG) enzyme which works to hydrolyze the glycosidic bonds of galacturonic acid (a component of pectin), the pectin methyl esterase (PME) enzyme which functions to hydrolyze the methyl-ester bonds of galacturonic acid which make up pectin and enzymes. β -D-galactosidase plays a role in removing the galactosyl group in pectin (Paniagua et al., 2014; Payasi et al., 2009; Sharma et al., 2015).

Conclusion

Based on the research that has been done, it can be concluded several things as follows:

1. Storage duration affects the crystal guava fruit's vitamin C and total soluble solids (TSS) content.
2. The level of fruit ripeness affects the crystal guava fruit's vitamin C and TSS content.
3. There is an interaction between the duration of storage and the level of ripeness on the vitamin C content of crystal guava fruit
4. Treatment with the best results:

- a. The combination of treatments to produce crystal guava fruit with a high vitamin C content was unripe fruit which was stored for 10 days.
- b. The best treatment to produce crystal guava fruit with the highest TSS was very ripe fruit and fruit stored for 5 days.
- c. Based on vitamin C, TSS, and the fruit's physical condition, the best crystal guava fruit was the unripe ones that is stored for 10 days

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Sari WK · Malik PA

The effects of application of biochar from oil palm empty fruit bunches on chemical properties of ultisols and the growth of cacao seedlings

Abstract. It is necessary to redevelop cacao commodity due to the decrease of cacao planting areas in Indonesia since the last decade. One of the ways is by providing a growing medium to produce cacao seedlings with good quality, such as by adding ameliorant i.e., oil palm empty fruit bunch (OPEFB) biochar to marginal soil of Ultisols. This research aimed to study and obtain the best OPEFB biochar dose to improve the chemical properties of Ultisols and the growth of cacao seedlings. This research was conducted in the Experimental Field of the 3rd Campus Andalas University from August 2021 until February 2022 using a Completely Randomized Design (CRD) with 5 treatments of OPEFB biochar (0, 60, 90, 120, 150 tons/ha) and 4 replications, each experimental unit consisted of two plants, so that 40 plants were prepared in total. Data obtained were analyzed using F Distribution Test at 5% and further analyzed using Duncan's New Multiple Range Test (DNMRT) for statistically significant results. The results showed that the application of OPEFB biochar at 120 tons/ha was the best dose to give significant results on the chemical properties of Ultisols (pH, organic carbon, total nitrogen, available phosphorus, exchangeable potassium) and several growth variables of cacao seedlings (stem diameter, leaf length, leaf width, shoot dry weight, and shoot-root ratio).

Keywords: Ameliorant · Biochar · Nursery · Oil palm empty fruit bunch · Ultisols

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Sari WK^{1*} · Malik PA²

¹Department of Estate Crops Cultivation, Faculty of Agriculture, Andalas University, Dharmasraya Campus 27573, West Sumatra, Indonesia

²Agrotechnology Undergraduate Programme, Faculty of Agriculture, Andalas University, Dharmasraya Campus 27573, West Sumatra, Indonesia

*Correspondence: wulanks@agr.unand.ac.id

Introduction

Cacao (*Theobroma cacao* L.) produces cocoa as Indonesia's leading export commodity, and Indonesia is the third largest exporter of cocoa beans in the world. However, this decade the area of cacao plantations in Indonesia tended to decrease, so an effort is needed such as by supplying high-quality cacao seedlings, which require a planting medium with ideal physical, chemical, and biological properties for its growth. But not all regions in Indonesia have an ideal soil type as a planting medium, like Ultisols, which is a marginal soil but can still be utilized if it is treated first.

According to Hardjowigeno (2003), Ultisols are soils that contain low organic matter and the structure is less stable, so it is sensitive to erosion. The nutrient content in Ultisols is generally low because of intensive alkaline leaching. At the same time, the organic matter is low because the decomposition process runs fast and some are carried away by erosion. Prasetyo and Suriadikarta (2006) added that Ultisols have some limitations when used in agriculture due to the unfavorable physical and chemical properties of the soil, such as high soil acidity (an average soil pH < 4.5), high aluminum saturation, poor macronutrient content especially P, K, Ca and Mg and low organic matter content.

Consequently, it is important to improve the properties of Ultisols by adding soil amendments (ameliorant). Ameliorants can be as inorganic or organic materials, like biochar. Handani (2017) revealed that ameliorant (biochar) can increase the Cation Exchange Capacity (CEC) in Inceptisols, which is able to support plant growth. The utilization of biochar can be an option for soil management for restoring and improving the quality of degraded soil fertility or critical agricultural land (Glaser, 2001). According to Lehmann and Joseph (2009), biochar is produced from organic materials burned imperfectly (pyrolysis) or without oxygen at high temperatures. Biological charcoal formed from this combustion will produce activated carbon. The benefits of biochar are having a high affinity for nutrients and being persistent in the soil. These properties can be used to solve problems in Ultisols with a low affinity for macronutrients.

One of the organic materials that can be used as biochar is Oil Palm Empty Fruit Bunch

(OPEFB). Mandiri (2012) stated that processing 1 ton of palm oil produces solid waste as empty fruit bunches of 23 % (230 kg). Because of the abundant amount of OPEFB waste and the probability of environmental pollution, it is necessary to utilize OPEFB waste into biochar which can be used for a mixture of planting media as an ameliorant which is useful in improving the quality of the planting media, such as in growing media using Ultisols.

Ismail and Basri (2011) revealed that the addition of biochar to agricultural soil can improve soil structure, retain water and soil from erosion because it has a larger surface area, enrich organic carbon, and increase soil pH. The research of Darmawan and Harjadi (2013) evidence that the application of biochar can overcome the lack of soil organic matter on newly opened paddy fields. Gusmailina et al. (2015) also declare the effect of biochar on tea plantations for 10 years when sprinkled around the tea plants as 100 g each, it turned out to have a high growth effect and increased production by 40% compared to plants that were not sown with biochar.

Specific for biochar from OPEFB, Irwanto (2019) who apply OPEFB biochar to Ultisols at a dose of 25 g/polybag was able to increase the soil pH by 0.15 to 4.94 and a dose of 50 g/polybag increased the soil pH by 0.79 to 5.45. Based on the above framework, the objective of the present research was to study and obtain the best OPEFB biochar dose to improve the chemical properties of Ultisols and the growth of cacao seedlings.

Materials and Methods

This research was carried out on August 2021 until February 2022 at the Experimental Field of the 3rd Campus, Andalas University, Dharmasraya, and analysis of soil chemical properties was carried out at the Laboratory of Tropical Fruit Research Institute, Aripian, Solok, West Sumatra. The materials used were cacao seeds BL 50 clone, Ultisols as planting medium, polybags 35 cm x 15 cm, fertilizer (Urea, TSP, KCl), oil palm empty fruit bunches (obtained from PT. Bina Pratama Sakato Jaya, Kiliran Jao, Sijunjung), paper envelope and label. While the equipment was a biochar maker, hoe, watering can, shovels, tape measure, vernier calipers, buckets, paranet 75%, scale, camera, and stationery.

The present research was arranged based on a Completely Randomized Design (CRD) with Oil Palm Empty Fruit Bunch (OPEFB) biochar dose as a treatment that consisted of 5 levels, i.e.: P0: 0 ton/ha biochar OPEFB (control); P1: 60 tons/ha OPEFB biochar (100 g/polybag); P2: 90 tons/ha OPEFB biochar (150 g/polybag); P3: 120 tons/ha OPEFB biochar (200 g/polybag); P4: 150 tons/ha OPEFB biochar (250 g/polybag). It was repeated four times to obtain 20 experimental units, and each of them consisted of 2 cacao plants in different polybags, so a total of 40 samples.

The research was preceded by cleaning the area and installing a shade that used paranet 75 %. The next step was to make oil palm empty fruit bunches (OPEFB) biochar with a self-designed tool (biochar maker): the OPEFB used are dry and chopped. Coconut shell as fuel which burned immediately covered with a biochar maker. Then, the OPEFB is piled up around the tool at about half of its height and waits until white smoke comes out (\pm 5 minutes) from the chimney. After that, the OPEFB waste that is being burned is turned upside down until it is evenly burned. After it turns black, it is separated between the biochar and the tool to prevent further combustion. Then doused with water, after cold biochar ready to use.

The planting medium used was Ultisols which is filled in a 35 cm x 15 cm polybag (capacity of 5 kg) plus a dose of OPEFB biochar according to treatment of each polybag, and then cacao seeds (BL 50 clones) were planted there. The distance between experimental units was 30 cm, and each consisted of 2 plants in different polybags spaced out 10 cm. Plant maintenance, namely watering twice a day, replacement of dead seedlings or grow abnormally (until a month after planting), weeding, control of pests and diseases and fertilization was applied a month after planting with a dose of 5 g urea/plant, 5 g TSP/plant and 4 g KCl/plant.

Observation variables: analysis of soil chemical properties (before & after treatments and growing cacao seedlings for 16 weeks), the parameters analyzed were soil pH, organic carbon by Walkey and Black method, total nitrogen by destruction method, available phosphorus by the Bray II method, and exchangeable potassium by the 1 M ammonium acetate washing method pH 7.0 (Soil Research Institute, 2009). Besides that, observations of plant growth variables on cacao seedlings

consisted of seedling height (cm), stem diameter (mm), number of leaves, leaf length and width (cm), longest root (cm), shoot and root dry weight (g), as well as shoot root ratio by comparing the shoot dry weight with root dry weight.

The data obtained from observing soil and plant samples were tested using F Distribution Test (ANOVA) at 5 % level and further analyzed using Duncan's New Multiple Range Test (DNMRT) for statistically significant results.

Results and Discussion

Soil Chemical Properties before Treatment OPEFB Biochar. Table 1 shows soil pH of Ultisols used as the planting medium was acid, organic carbon, total nitrogen, and exchangeable potassium was very low, and available phosphorus was low. According to Hardjowigeno (2003), Ultisol is derived from very acidic parent materials and occurs in advanced weathering soils. It also contains low organic matter and the structure is unstable.

Table 1. Several chemical properties of Ultisols before treatment OPEFB Biochar.

Parameters	Value	Criteria*
pH H ₂ O	4.53	Acidic
C-organic (%)	0.96	Very low
N-total (%)	0.17	Very low
Available-P (ppm)	2.49	Low
Exchangeable potassium (me/100g)	0.06	Very low

*) Source: Soil Research Institute (2009)

Fitriatin et al. (2014) stated that Ultisols problems are acidity soil, low organic matter and low macronutrients. Advanced weathering of Ultisols can form high amounts of hydrous oxide clay Fe & Al and react with P to form hydroxyls which are difficult to dissolve so that P is less available in the soil. The value of N-total in this research was 0,17 % (very low criteria) due to it being associated with the C-organic content (0,96 %) in Ultisols. They have a close connection, low C-organic resulted in low N-total because organic matter is one nitrogen source in the soil (Syahputra et al., 2015).

Exchangeable potassium is also in very low criteria (0.06 me/100g). In line with Mulyani et al. (2010), Ultisols is advanced weathering soil tends to be poor in potassium. Besides that, rainfall influences exchangeable potassium value, where

some areas in Indonesia have heavy rainfall, causing high washing cations base that affects low exchangeable potassium and the soil becomes acidic.

The Effects of Application OPEFB Biochar on Several Chemical Properties of Ultisols

Soil pH. The application of OPEFB biochar to Ultisols generates a significant effect on the soil pH. A significantly different effect of OPEFB biochar at 120 tons/ha compared with 0 – 90 tons/ha, while at 150 tons/ha only significantly different with without treatment (Table 2).

Table 2. Ultisols pH after Treatment OPEFB Biochar as planting media in cacao seedlings.

Treatment	pH*	Criteria**
P0: 0 ton/ha	4.60 a	Acidic
P1: 60 tons/ha	5.06 ab	Acidic
P2: 90 tons/ha	5.15 b	Acidic
P3: 120 tons/ha	6.02 c	Slightly acidic
P4: 150 tons/ha	5.77 bc	Slightly acidic
CV = 9.20 %		

*) Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

**) Source: Soil Research Institute (2009)

Generally, OPEFB biochar is good for Ultisols, it is capable of increasing pH. In line with Nigussie et al. (2012); Putri et al. (2017); and Irwanto (2019) that biochar is the potential to improve soil chemical properties, such as increasing the soil pH. Fadilah (2020) also revealed that biochar at a dose of 60 tons/ha is capable to increase Ultisols pH (3.93 to 4.25) as a planting media in Robusta coffee seedlings.

In this research, soil pH was increased in line with adding biochar doses of up to 120 tons/ha, but it decreased after reaching 150 tons/ha. It is similar to the research results of Handani (2017) that the application of OPEFB biochar in a huge amount causes an increase in pH is not optimal. This suspected because the negative biochar particles will bind cations including H⁺ ions as a reason for soil acidity. This is confirmed by Sujana (2014) that biochar particle is negatively charged so highly the ability to bind cations.

Organic Carbon. ANOVA results showed that the application of OPEFB biochar significantly affected Ultisols organic carbon (Table 3).

Table 3. C-organic after treatment OPEFB Biochar as planting media in cacao seedlings.

Treatment	C-organic (%)*	Criteria**
P0: 0 ton/ha	0.89 a	Very low
P1: 60 tons/ha	0.95 a	Very low
P2: 90 tons/ha	1.13 a	Low
P3: 120 tons/ha	1.53 b	Low
P4: 150 tons/ha	2.43 c	Low
CV = 11.16 %		

*) Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

**) Source: Soil Research Institute (2009)

The effects of biochar from oil palm empty fruit bunch at a dose of 0 – 90 tons/ha were not significantly different from each other, whereas it was significantly different if compared to 120-150 tons/ha. The highest C-organic content (2.43 %) was found in the treatment OPEFB biochar at 150 tons/ha. In this research, the increase of organic carbon is directly proportional to the increase in biochar dose. Similar results with Bella (2020), where the application of biochar at doses of 0, 20, 40, and 60 tons/ha resulted in C-organic content of 0.48 %, 0.75 %, 1.01 %, and 1.31 %, respectively. This happens because of the high carbon organic in biochar. According to Putri et al. (2017), OPEFB biochar has an organic carbon content ranging from 6-30 %. In line with Fadilah (2020) reported that OPEFB biochar has C-organic content of 9.65 % and Wahyuni et al. (2021) that OPEFB biochar contains C-organic by 22 %.

According to Lehmann (2007) biochar is able to bind CO₂ obtained from the incomplete combustion process (pyrolysis) and it is able to retain carbon so that it does not return to the atmosphere. This is in accordance with Widiastuti and Maria (2016) that biochar is resistant in soil because it contains high carbon (C) and is weather resistant so it is stable for decades in the soil. Biochar is better as a soil conditioner than compost because it much longer in storing nutrients to improve the physico-chemical properties of the soil which functions as a carbon storage (Sarwono, 2016).

Total Nitrogen. Table 4 shows the application of OPEFB biochar able to increase N-total content up to 107.14 % in Ultisols and it was significantly different results. Significantly different results were obtained on the treatment of OPEFB biochar at a dose of 120 and 150 tons/ha, i.e., 0.20 % and 0.29 % N-total.

Table 4. N-total after Treatment OPEFB Biochar as planting media in Cacao Seedlings.

Treatment	N-total (%) [*]	Criteria ^{**}
P0: 0 ton/ha	0.14 a	Low
P1: 60 tons/ha	0.14 a	Low
P2: 90 tons/ha	0.14 a	Low
P3: 120 tons/ha	0.20 b	Low
P4: 150 tons/ha	0.29 c	Moderate
CV = 9.25 %		

^{*}) Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

^{**}) Source: Soil Research Institute (2009)

The increase of N-total in line with the enhancement of C-organic content (Table 3). Sukaryorini et al. (2016) state that increased C-organic enhances the population of microorganisms, followed by the enhancement of N nutrient. An increase in N-total soil was also found by Khasanah et al. (2020), where the application of OPEFB biochar 0.5 kg was able to increase N-total Ultisols to 0.40 %, and the dose of 1 kg increases the total-N content to 0.47 %, in comparison with N-total in Ultisols without treatment of 0.26 %. Fadilah (2020) reported that adding OPEFB biochar on Ultisols at a dose of 60 tons/ha can increase the N-total to 0.19 %.

Herlambang et al. (2021) stated that biochar can increase soil nutrient availability, microbe activity, organic matter, water retention, and retain N element because biochar effectively absorbs NO₃⁻ and NH₄⁺ ions. Putri et al. (2017) revealed that biochar has a high capacity to water retention, so it can maintain N nutrient so not leaching and is available for plant growth. Supported by Fadilah (2020) that the addition of OPEFB to Ultisols can increase the water level to 10 % and increase the index porosity to 4 %.

Available Phosphorus. Treatment of OPEFB biochar results significantly different in available P on every dose of biochar, except at 60 tons/ha, which was not significantly different without treatment (0 ton/ha) and 90 tons/ha (Table 5). In the present research, the application of OPEFB biochar can increase available P by 22.56 % - 275.29 %, and this is in line with the results of Herlambang et al. (2021) that the application of biochar on Ultisols, which is acidic, can increase available P of 4.9 % - 142.9 %. It was also obtained by Sitompul (2020) that gift OPEFB biochar on Ultisols at a dose of 90 tons/ha can increase available P 17,52 ppm (very high criteria).

Salawati et al. (2016) added that applying biochar on acidic soil can increase available P content of 277.08 %, with a dose of 15 tons/ha increasing available P content to 47.55 ppm.

Table 5. Available P after treatment OPEFB Biochar as planting media in cacao seedlings.

Treatment	Available P (ppm) [*]	Criteria ^{**}
P0: 0 ton/ha	15.38 a	Very high
P1: 60 tons/ha	18.85 ab	Very high
P2: 90 tons/ha	22.24 b	Very high
P3: 120 tons/ha	36.04 c	Very high
P4: 150 tons/ha	57.72 d	Very high
CV = 12.95 %		

^{*}) Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

^{**}) Source: Soil Research Institute (2009)

Atkinson et al. (2010) revealed the increase of available phosphorus because biochar contains P elements that are released and dissolved in soil solution. Besides that, an increase of available P in the present research was suspected because of the application of TSP fertilizer that collaborate with P contained in OPEFB biochar that increases soil capacity to hold water so that a given P will also increase or not leach. Karbeka et al. (2022) also asserted an increase in available P because of the release of Al-P and Fe-P due to the OPEFB biochar application on Ultisols. It is able to release P adsorption by Al-P and Al-Fe through anion exchange, organic acid anions will replace the position of P elements which are fixed by Al and Fe so that the available P element content in the soil will increase.

Exchangeable Potassium. Application of OPEFB biochar gave a significantly different in exchangeable potassium (K) of Ultisols. There was an increase in exchangeable K content in line with the increase in the dose of biochar given, although at 120 and 150 tons/ha not significantly different (Table 6). It was conformable by Handani (2017) that there was an increase of exchangeable K along with an increase of OPEFB biochar doses, i.e., 0; 5.3; 10.6; and 21.2 g/plant produce exchangeable K was 0.18; 0.23; 0.25; and 0.28 me/100g, respectively.

It was because of the K content in the OPEFB biochar, as confirmed by Fadilah (2020) that OPEFB biochar contained 1.44 me/100g exchangeable potassium. This is in accordance with the opinion of Lehmann and Joseph (2009)

that biochar application contributes to an increase in soil charge. Bakar et al. (2015) stated that biochar has a negative charge to retain soil cations such as K^+ ions. In line with the opinion of Sujana (2014), biochar produced from the pyrolysis process creates negatively charged particles that have a greater ability to absorb soil cations, including K^+ . In addition, another factor that affects the exchangeable K is the C-organic content of Ultisols (Table 3). The increase of K balance in the soil depends on the C-organic content because C-organic can control K availability as indicated by a positive correlation with soil resistivity (Nursyamsi et al., 2007).

Table 6. Exchangeable K after treatment OPEFB Biochar as planting media in cacao seedlings.

Treatment	Exchangeable K (me/100g)*	Criteria**
P0: 0 ton/ha	0.76 a	High
P1: 60 tons/ha	2.26 b	Very high
P2: 90 tons/ha	3.00 c	Very high
P3: 120 tons/ha	3.69 d	Very high
P4: 150 tons/ha	4.16 d	Very high
CV = 15.19 %		

*) Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %

**) Source: Soil Research Institute (2009)

The Effects of The Application OPEFB Biochar on The Growth of Cacao (*T. cacao* L.) Seedlings

Seedling Height. Table 7 shows the effect of biochar from oil palm empty fruit bunches was not significantly different on the variable of seedling height. The height of cacao seedlings with 150 tons/ha of OPEFB biochar increased by 39.99 % compared to without biochar (0 ton/ha). However, overall the height growth has exceeded the minimum height of cacao seedlings at 3-6 months that must be 20-30 cm (Indonesian Coffee and Cocoa Research Institute, 2010).

Table 7. Height of cacao seedlings at 16 weeks after planting (WAP) after application OPEFB Biochar.

Treatment	Seedling Height (cm)
P0: 0 ton/ha	39.83
P1: 60 tons/ha	47.84
P2: 90 tons/ha	50.34
P3: 120 tons/ha	50.98
P4: 150 tons/ha	55.76
CV = 14.66 %	

Conversely, Syarifain et al. (2022) who used several types of soil ameliorant in oil palm nurseries obtain significantly different effects on the variable of plant height. The growth of plant height is influenced by available nutrients in growing media, especially N nutrient which functions in acceleration of cell division. Table 4 shows the total nitrogen in all treatments was categorized into low criteria, except 150 tons/ha was a moderate criterion. This is a strong reason why the result was not significantly different, where fewer nutrients in the growing medium will affect the plant height.

Growth of plant height goes on vegetative phases that relate to three important processes, i.e., cell division, elongation, and differentiation. Those processes need carbohydrates that compound with N element at the growing point that influence the increase of plant height. The optimal cell division must be supported by N availability which plays a role for stimulate overall growth, especially stem growth and if the availability of it is a low criterion so plant growth will slowdowns (Mardianto, 2014).

Stem Diameter. Based on analyzed of variance shows that the application of OPEFB biochar gives a significant effect on the stem diameter of the cacao seedling. Dose 120 and 150 tons/ha were not significantly different, but they were significantly different when compared to the dose of 0 and 60 tons/ha. The largest stem diameter (11.0 mm) was found in the treatment of 120 tons/ha OPEFB biochar (Table 8). According to the standard growth of cacao seedlings, the stem diameter in this research has fulfilled it, where a minimum stem diameter of > 6 mm at seedlings of 3-6 months (Indonesian Coffee and Cocoa Research Institute, 2010).

Table 8. Stem diameter of cacao seedlings at 16 WAP after application OPEFB Biochar.

Treatment	Stem Diameter (mm)
P0: 0 ton/ha	10.11 a
P1: 60 tons/ha	10.25 a
P2: 90 tons/ha	10.54 ab
P3: 120 tons/ha	11.20 b
P4: 150 tons/ha	11.15 b
CV = 5.32 %	

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

The growth of stem diameter is absolutely influenced by nutrient availability in the soil or

growing media, such as potassium (K). Potassium nutrient plays a role in accelerating meristematic growth, especially stem diameter and strengthening plant. The content of exchangeable potassium in this research shows in Table 6, a growing medium without treatment (0 ton/ha OPEFB biochar) containing 0.76 me/100g exchangeable K (high criterion), while the exchangeable K contained in the growing medium at a dose of 150 tons/ha OPEFB biochar reached 4.16 me/100g (very high criterion). This is directly proportional to the increase of stem diameter of cacao seedlings in the present study.

Besides the nutrient availability, stem diameter growth is also influenced by soil pH. According to analysis results of soil pH (Table 2) the pH at a dose of 120 and 150 tons/ha OPEFB biochar were 6.02 and 5.77, respectively. It is related to Susanto (1995) opinion that cacao will grow optimally at soil pH 6.0 – 7.0 because soil pH influences ability of cacao plant to absorb nutrients. On the other hand, Hayati et al. (2021) stated that although there was an increase in soil pH in Ultisols, it was still relatively low because the presence of Al and Fe ions predominate will make nutrient uptake becomes decreased.

Number of Leaves. Table 9 shows no significant effect of OPEFB biochar on the leaves number of cacao seedlings aged 16 weeks after planting (WAP). It was increased by 26.47 % at a dose of 120 tons/ha OPEFB biochar compared to without biochar (0 ton/ha). This results in line with Yonedi (2021) that the application of OPEFB biochar not a significantly different effect on the leaves number of rubber seedlings. Also, by Siboro (2018) who gift biochar on Ultisols as growing medium on the growth of oil palm in the main nursery, it was no significantly different effect to the leaves number.

Table 9. Leaves number of cacao seedlings at 16 WAP after application OPEFB Biochar.

Treatment	Number of Leaves
P0: 0 ton/ha	23.80
P1: 60 tons/ha	26.10
P2: 90 tons/ha	27.30
P3: 120 tons/ha	30.10
P4: 150 tons/ha	28.00
CV = 15.30 %	

In this study, it was suspected that there was a connection between the plant height and leaves number, where obtained not significantly different results on the both of growth variables.

This opinion is supported by Haryadi (2015) that increase plant height will stimulate the formation leaf young that grows on stems.

Pangaribuan (2001) opine that besides depending on age plant, the increase of leaves number is genetic characteristic of each plant. Besides that, the development of cacao leaves affected by speed of production leaves that depend on local climate and soil conditions. On fertile soil, leaves will fast open so that the more effectively do function as photosynthesis place and respiration tools.

Leaf Length and Width. Results of ANOVA shows that OPEFB biochar has a significant effect on the leaf length and width of cacao seedlings (Table 10). The longest leaf length (29.33 cm) was obtained at a dose 120 tons/ha which is significantly different with the other four doses. Meanwhile, for leaf width at doses of 90 and 120 tons/ha were significantly different with 0 ton/ha (control), while doses of 60 and 150 tons/ha were no significantly different to each other. Such results are certainly related with the chemical properties of Ultisols which are affected by the OPEFB biochar, specifically N nutrient. Ulfa (2018) stated that the nutrient that has most influence on leaf growth and development is N.

Table 10. Leaf length and width of cacao seedlings at 16 WAP after application OPEFB Biochar.

Treatment	Leaf Length (cm)	Leaf Width (cm)
P0: 0 ton/ha	23.66 a	8.39 a
P1: 60 tons/ha	24.16 a	8.78 ab
P2: 90 tons/ha	25.39 a	10.06 b
P3: 120 tons/ha	29.33 b	9.98 b
P4: 150 tons/ha	25.59 a	9.68 ab
CV = 8.95 %		CV = 8.96 %

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

High levels of N generally produce longer and larger leaves because it is used in cell division and elongation. Nitrogen is used to form amino acids where it will be converted into proteins. Nitrogen is also needed to form important compounds, such as chlorophyll, nucleic acids and enzym. Therefore, nitrogen is needed in relatively large quantities at each stage of plant growth, especially the vegetative growth including on the formation of shoots and the growth of stems and leaves (Novizan, 2005).

The result on the variable of leaf width was similar with Jelvina (2019), where the application of OPEFB biochar had a significant effect on the leaf width of oil palm in the main nursery. Treatment of OPEFB biochar at 90 tons/ha has provided nutrients for the growth of leaf width, due to application of OPEFB biochar is assist the mineralization of N element. According to Nguyen et al. (2017), the application of biochar can increase soil moisture and pH, thereby stimulating N mineralization and nitrification processes which cause plant uptake to increase. Biochar increases the inorganic N required for plant assimilation by increasing retention and reducing the impact of N leaching.

Lakitan (2010) stated that nitrogen affects the formation of new cells, phosphorus affects activating enzymes, and potassium influences the development of meristem tissue which affect the length and width of leaves. In line with Dhani et al. (2013), the formation of leaves in plants is greatly influenced by the availability of nitrogen, phosphorus and potassium nutrients in the growing medium. These elements play a role in the formation of new cells and main components of organic compounds in plants such as amino acids, nucleic acids, chlorophyll, ADPs and ATPs. Tables 4, 5 and 6 show an increase in those nutrient contents in line with the additional dose of OPEFB biochar given. It is a fundamental reason for the significant effect of the application of OPEFB biochar on the leaf length and width of cacao seedlings.

Root Length. Table 11 shows the application of OPEFB biochar has no significantly different effect on the root length of cacao seedlings, while the root length with 120 tons/ha of OPEFB biochar increased by 1.99 % compared to without biochar. This result was similar with Fadilah (2020) that the application of OPEFB biochar also did not have a significant effect on the root length of the robusta coffee seedlings.

Table 11. Root Length of Cacao Seedlings at 16 WAP after Application OPEFB Biochar.

Treatment	Root Length (cm)
P0: 0 ton/ha	41.70
P1: 60 tons/ha	42.35
P2: 90 tons/ha	38.80
P3: 120 tons/ha	42.53
P4: 150 tons/ha	41.70
CV = 15.13 %	

The results are not significantly different on the variable of root length presumably because of the root growth had reached the lowest point on the polybag sized 35 x 15 cm so that roots could not grow more because the limitations of growing spaces. According to The Ministry of Agriculture (2011), at the beginning of seed germination, the taproot grows rapidly ranging 1 cm in length (1 week old) to 25 cm (3 months old). After that, speed development will down and reach 50 cm.

Shoot-Root Dry Weight and Shoot-Root Ratio. ANOVA results showed a significant effect on the shoot dry weight of cacao seedlings at 16 WAP due to the application of OPEFB biochar. Treatments of P3 and P4 (doses of 120 and 150 tons/ha) were significantly different with doses of 0, 60, and 90 tons/ha (P0, P1, and P2), and the heaviest shoot dry weight (41.71 g) was obtained at a dose of 150 tons/ha. On the contrary, the OPEFB biochar effect was not significantly different for root dry weight and an increased of 25.89 % was found at a biochar dose of 150 tons/ha compared to without biochar (Table 12).

Shoot dry weight is the main indicator of the accumulation of dry matter (photosynthate) in above-ground which is strongly influenced by the growth of stems and leaves. In line with Sahroni et al. (2008), shoot dry weight is the accumulation of photosynthetic results which cause growth such as increasing plant height and leaf area.

Table 12. Shoot-root dry weight and shoot-root ratio of cacao seedlings at 16 WAP after application OPEFB Biochar.

Treatment	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot-Root Ratio
P0: 0 ton/ha	18.17 a	5.87	3.09 a
P1: 60 tons/ha	21.64 a	6.05	3.58 a
P2: 90 tons/ha	29.46 b	4.10	7.18 b
P3: 120 tons/ha	39.28 b	6.23	6.30 b
P4: 150 tons/ha	41.71 b	7.39	5.64 b
CV = 13.91 %		CV = 13.39 %	CV = 22.18 %

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Duncan's multiple range test at the level of 5 %.

Sitompul and Guritno (1995) revealed that calculating plant dry weight is important because dry weight is an indicator of plant metabolism. The dry weight can represent the results of plant metabolites. It is also used as an indicator of plant growth because dry weight shows organic compounds that are translocated to all plant organs.

Roidi (2016) declares that the root dry weight depends on the uptake of nutrients and the root length. If photosynthesis goes well, the root will grow well too, followed by enhancement of heavy root dry. Root dry weight is the accumulation of organic matter and is closely related to the growth of root length. Table 11 shows that OPEFB biochar did not significantly effect on the variable of root length and it was a strong reason why in the variable of root dry weight that not significantly different also, because both of it was closely related.

Gardner et al. (1991) revealed that root growth includes root elongation and widening which are influenced by media and environmental factors. The application of biochar can improve soil structure, organic matter will improve the soil properties so creating a better environment for roots to absorb more nutrients. Organic matter contributes to pore space and higher water-holding capacity in the root zone resulting in heavier and stronger roots.

Root hairs are the part of the root that is most active in absorbing nutrients and water. The more root hairs are formed, the greater amount of nutrients and water absorbed by the plant roots. The heaviest root dry weight was obtained on the treatment of P4 (150 tons/ha), presumably related to the organic carbon content on that treatment, namely 2.34 % (Table 3).

On the variable of shoot root ratio, it was shown that the application of OPEFB biochar had a significant effect. Its value was obtained from the comparison of shoot dry weight and root dry weight. This is necessary to know the direction of photosynthate allocation and growth of cacao seedlings, whether to the shoot or root. That is related to the opinion of Sari (2013) that a value of shoot root ratio more than one (> 1) indicates the growth of the plant is more towards the shoot and vice versa if less than one (< 1) indicates the growth of the plant is more towards the roots.

In this study, the value of shoot root ratio whole > 1 indicates that photosynthate allocation and growth of cacao seedlings are more towards the shoot. Moreover, in the vegetative phase,

roots function as nutrient absorption so that the growth of the shoot is greater than the root and dry weight through photosynthesis is more translocated to the shoot rather than the root.

Conclusion

Based on the results and discussion, it can be concluded that the application of biochar from oil palm empty fruit bunches at 120 tons/ha (equivalent to 200 g/polybag) was the best dose to give significant results on several chemical properties of Ultisols (pH, organic carbon, total nitrogen, available phosphorus, exchangeable potassium) and several growth variables of cacao seedlings (stem diameter, leaf length and width, shoot dry weight, and shoot-root ratio).

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Setiadi A · Hosang EY

Characteristics of the moringa mother tree in the population of East Flores, East Nusa Tenggara

Abstract. The moringa plant (*Moringa oleifera* Lam.) is a member of the Moringaceae family that grows in Indonesia. The use of moringa plants in food production, animal feed, pharmaceutical manufacturing, and the cosmetics industry are just a few of their many advantages. The development of moringa plants in Indonesia has not been carried out because it is still constrained by the absence of a mother tree and a source of moringa seed gardens. This study aims to identify morphological characters and production potential in two populations of moringa plants. The research was conducted in August 2021 in East Flores District, East Nusa Tenggara Province. The research was conducted by determining the selected mother tree (SMT), observing morphological characters, and seed production. Exploration of selected parent trees was carried out using a survey method on two moringa plant populations in Tiwatobi and Harubala villages. Observations of plant morphological characters were carried out on quantitative and qualitative characters. The production potential is carried out by estimating the production of moringa seeds in each population. The results showed that there were 19 SMTs in the first population and 29 SMTs in the second population. 27 morphological characters have been observed, with the potential for seed production in each population of 314,554 seeds and 529,538 seeds per year.

Keywords: Moringa · Morphology · Mother tree · Population · Seed

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Setiadi A^{1*} · Hosang EY²

¹ Research Center for Horticultural and Estate Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta Bogor, Cibinong, Bogor 16915, West Java, Indonesia

² Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta Bogor, Cibinong, Bogor 16915, West Java, Indonesia

*Correspondence: adi.setiadi@brin.go.id

Introduction

Originally from southern Asia, the Moringa plant (*Moringa oleifera* Lam.) is a member of the Moringaceae family that can be found in northern India, Pakistan, Bangladesh, and Nepal (Roloff et al., 2009). This plant can grow in the lowlands to highlands in the tropics and subtropics. Furthermore, the moringa tree is known for its fast growth rate and ability to regenerate quickly after pruning or damage (Trigo et al., 2020). This plant can grow in a wide range of soil types and climatic conditions, including areas with low rainfall and poor soil fertility (Alia et al., 2022). Moringa plants can be found in Sumatra, Java, Sulawesi, and Nusa Tenggara in Indonesia (Roshetko et al., 2017).

Moringa plants are also known as "*the miracle tree*" because they have a high nutritional content, and are widely used as a functional food (Moyo et al., 2011; Muflihatin et al., 2021). Moringa leaves are highly nutritious and are known to contain a range of vitamins and minerals, making them a valuable food source in areas where malnutrition is prevalent (Dhawi et al., 2020; Ojo, 2019). The moringa leaf consists of high amounts of protein, essential amino acids, and antioxidants, further contributing to its value as a plant species (Abimbola & Olabisi, 2020). Moringa leaves have a content of vitamin C 4 times greater than oranges, vitamin A 10 times greater than carrots, calcium content 17 times more than milk, protein nine times more than yogurt and potassium 17 times greater than bananas, and iron content 25 times greater than spinach (Rockwood et al., 2013; Utami et al., 2022). In addition, various parts of the plant have been used for medical purposes due to their antimicrobial (Raju & Kei, 2022) and antioxidant properties (Bawadekji et al., 2019). Moringa leaves include quercetin and kaempferol, which have anti-diabetic and antioxidant properties (Siddhuraju & Becker, 2003; Gupta et al., 2012) frequently used parts are roots, bark, sap, leaves, pods, flowers, seeds, and seed oil. Moringa leaves' bioactive content can help people overcome heart disease, hypertension, insulin resistance, and cancers (Dhakad et al., 2019).

Moringa plants are found across Indonesia, particularly in the eastern islands of Sulawesi and Nusa Tenggara. Plant growth is influenced by heredity/genetic factors as well as the environment. So, to cultivate moringa plants with high output, seeds that are genetically beneficial

in production and resistant to plant-disturbing organisms are required. The moringa mother tree's ability to adapt to different growing conditions, fast growth, and regeneration rate, deep taproot system for accessing water and nutrients, and its high nutritional and medicinal value make it an ideal plant species for a variety of purposes, including food security, health promotion, and environmental preservation (Alia et al., 2022). Unfortunately, no moringa plant variety has had high productivity (Suketi et al., 2016). Furthermore, characterization and inventory of moringa plant populations have not been regularly carried out in Indonesia to date, despite these activities being the initial step in plant breeding operations. The exploration of moringa plant characteristics and their potential uses is an important area of research, particularly in developing countries where food insecurity and malnutrition are pervasive issues (Dasat et al., 2020). As a result, efforts are required in Indonesia to explore, describe, and offer moringa plant seeds.

Materials and Methods

The research was conducted in August 2021 in East Flores Regency, East Nusa Tenggara Province (ENT). The material used is Moringa stands located in two different populations: Tiwatobi Village, Ile Mandiri District, and Harubala Village, Ile Boleng District, East Flores Regency. The tools used in this study were meters, digital cameras, *Global Positioning System* (GPS), calipers, ash-colored fabrics, labels, stationery, plastic bags, and digital scales.

Procedure. This research consists of several stages of activities, including:

Exploration and identification of the Moringa plant's mother tree. Due to its diverse and beneficial characteristics, the *Moringa oleifera* Lam. tree is a versatile and valuable plant species (El-Esawy et al., 2018). Moringa plant population exploration location was obtained based on information from the ENT Provincial Agriculture and Plantation Office and the East Flores Regency Agriculture and Plantation Office. The Moringa plant population used in this study has a minimum area of 0.25 Ha, is more than three years old, and is in the form of a bed according to Minister of Agriculture Decree Number 79/Kpts/KB.020/ 12/2020 concerning

Guidelines for Moringa Plant Production, Certification, and Seed Supervision.

Purposive sampling is used to select the mother tree. The selection of the parent tree is based on observations of superior moringa plant phenotypes, the absence of plant-disturbing organisms, and high seed production compared to moringa plants within the population. The selected parent tree is then recorded and marked using paint/iron plates, and its coordinates are recorded.

Observation of morphological characters of Moringa plants. The morphological characters observed include qualitative and quantitative characters of moringa plants regarding moringa descriptors (Angadi & Jagadeesha, 2018). As qualitative character qualities, the shape of the leaves, the tip shape of the leaves, the base shape of the leaflets, the form of the stem, the color of the stem, the surface of the stem, the color of the fruit, and the color of the seeds were all observed. Tree height, canopy length, stem diameter, leaf length, leaf breadth, petiole leaf length, leaf child length, leaf child width, pod length and pod diameter, and seed quantity are among the quantitative parameters recorded. Moringa plants are also taxed every year to determine annual seed output.

Moringa seed production taxation. Seed taxation is carried out by observing the number of productive branches/trees (PB, branches with pods ready for harvest of more than 30%), the average number of pods/trees (NP), and the average number of seeds/pods (NS). The number of moringa/tree seeds was calculated by sampling physiologically mature pods on one of the productive branches. Finally, the potential of seeds/trees/year (PS) is calculated using the formula:

$$PS = PB \times NP \times NS \times \text{Number of harvests in 1 year}$$

Results and Discussion

Moringa plant exploration. The Moringa plant is one of the people of ENT as a fence plant. Moringa plants are planted in small amounts in people's yards, therefore they are rarely found in large patches. Two populations of moringa plants were obtained in the form of costs based on the results of exploration in East Flores Regency, namely in Ile Mandiri District (08° 16'57.1", 123°

00'10.2") and Ile Boleng (08° 22'59", 123°17'11.3"). The moringa plant is referred to as marungge among the neighboring community. Moringa plants are used in ENT in the identical way as they are in other regions of Indonesia, namely as raw materials for vegetables and animal feed (Adli & Kuswanto, 2019).



Figure 1 The location of the Ile Boleng population's mother tree.

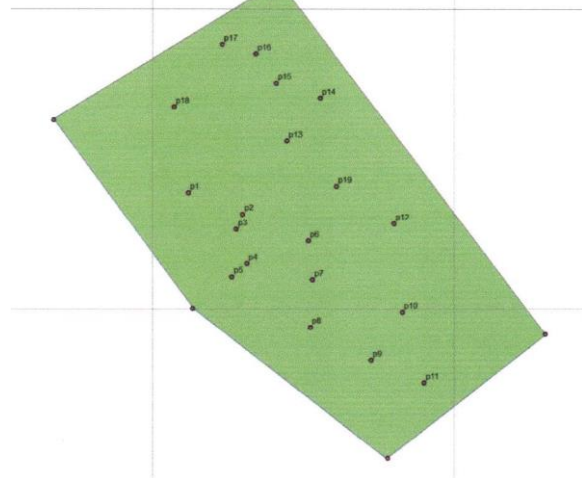


Figure 2. Location of selected mother trees in Ile Mandiri

The population of Ile Mandiri moringa plants is 9 m above sea level, planted in 2018 with an area of 0.5 Ha and 862 plants. The population of Ile Boleng moringa plants is at an altitude of 10 m above sea level, which was planted in 2013 with a land area of 0.5 Ha and a population of 627 plants. Both have a relatively flat topography (0-5%) and planting lengths that range from 1 m x 2 m to 3 m x 3 m to 4 m x 4 m. Following phenotypic selection, seed yield power of more than 3 kg/tree was achieved from as many as 19 trees in the Ile Mandiri

population and 29 trees in the Ile Boleng population as selected parent trees (SPT).

Characterization of diversity provides information on physical parameters such as leaf shape, harvest, plant height, and yield (Surahman et al., 2009). Moringa plant morphological characters observed include quantitative (Table 1) and qualitative (Table 2) characters. The population of Ile Mandiri moringa plants (7.59 m) has a greater average height when compared to the population of Ile Boleng plants (6.49 m). This height difference is thought to be because the planting distance in the Ile Mandiri population is tighter when compared to the Ile Boleng population. The leaf length of the Ile Boleng population (37 cm) is higher when compared to the Ile Mandiri population (35 cm) as well as the leaf width of the Ile Boleng population (25.10 cm) compared to the Ile Mandiri (25 cm). The same can be observed in the length and width of the leaflets and the length of the flower stalks. The length and width of the leaves of the Ile Boleng population (1.82 cm/1.42 cm) are higher than that of Ile Mandiri (1.80 cm/1.30 cm). Different results can be observed in stem diameter, petiole leaf length, pod length, pod circumference, number of seeds, and weight of 100 moringa seeds, where the Ile Mandiri population has a larger size when compared to the Ile Boleng population.

Table 1 The quantitative character of the Moringa plant population.

Parameter	Ile Mandiri	Ile Boleng
1. Height (meters)	7.59±1.44	6.49±0.38
2. Rod diameter (cm)	22.24±3.74	22.22±3.59
3. Leaf length (cm)	35.00±0.20	37.40±1.14
4. Leaf width (cm)	25.00±1.80	25.10±2.66
5. Leaf-child length (cm)	1.80±0.15	1.82±0.10
6. Leaf width (cm)	1.30±0.50	1.42±2.00
7. Panjang petiole (cm)	12.20±0.55	10.02±0.22
8. Flower stalk length (cm)	4.00±0.65	4.10±0.42
9. Pod length (cm)	40.72±1.77	40.33±0.70
10. Pod circumference (cm)	6.96±0.76	6.74±0.26
11. Number of seeds/pods	21.61±1.96	22.1±1.09
Weight: 100 seeds/tree (grams)	20.38±0.24	20.04±0.65

When viewed from the observed qualitative characters (Table 2), Ile Mandiri and Ile Boleng

populations have the same qualitative characteristics in observing stems, leaves, pods, and seeds. The leaves in both groups have a pointed leaf base with rounded leaf tips and dark green on the leaf surface, with a whitish-green underside. Both groups had green petiole leaves with a purple color (Figure 3). Moringa plant flowers are normally white with yellowish-white pistils and stamens, and neither population has any anthocyanin color. Moringa plants have flowers that include both male and female organs, allowing them to self-pollinate (Zhang et al., 2018). The pods of the second moringa plant population are dark green while young and turn light brown with brown seed color when harvested. There was no difference between the two populations in terms of physical characteristics. Its shows that there is a possibility that the seed source between the two populations has the exact seed source origin.

Table 2 Characteristics of the Moringa plant population.

Character	Qualitative character	
	Ile Mandiri	Ile belong
Leaf		
1. The shape of the tips of the leaves	Rounded corners	Rounded corners
2. The shape of the base of the leaf child	Tapering	Tapering
3. Leaf color	Green	Green
4. Lower color of the leaves	Whitish green	Whitish green
5. Leaf petiole color	Green with a red tinge	Green with a red tinge
Flower		
6. Flowering phase	One month	One month
7. Petals	White	White
8. Stamens color	Yellow	Yellow
9. Petal color	Yellowish white	Yellowish white
Pods and seeds		
10. Light pod color	Dark green	Dark green
11. Old pod color	Light brown	Light brown
12. Seed color	Dark brown	Dark brown

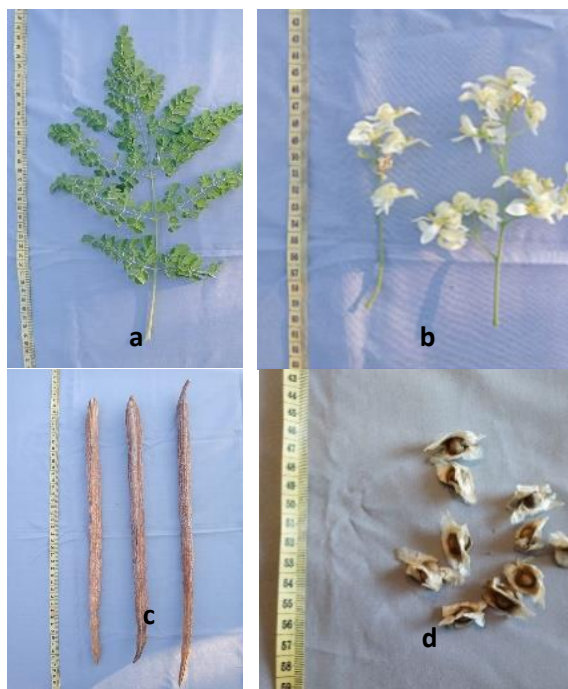


Figure 3. The character of leaves (a), flowers (b), fruits (c), and seed (d) of Moringa plants

Taxation of Moringa plant seed needs. Moringa has five harvest seasons, with two peak harvests in June and July. The flowering process of Moringa trees is heavily influenced by environmental conditions, particularly rainfall. Flowers fall off during the rainy season, decreasing pod production. The results of seed taxation (Table 3) show that the population of Ile Boleng (314,554 seeds/year) has a higher amount of seed production when compared to Ile Mandiri (529,538 seeds/year). The production of these two populations can be used as a seed source to propagate Moringa plants in ENT.

Table 3 Seed Taxation of Moringa plants per year

Population	Number of mother trees	Number of seeds/tree /year	Seed production (seeds/year)
Ile Mandiri	19	16,555	314,554
Ile Boleng	29	18,259	529,538

Conclusion

From the research that has been carried out, it can be concluded that:

1. The results of moringa plant exploration in East Flores Regency obtained two moringa

plant populations, namely the Ile Mandiri population (19 SPT) and Ile Boleng (29 SPT).

2. As many as 27 morphological characters have been observed, including qualitative and quantitative characters, which shows no difference in morphological characters between the two populations.
3. Based on taxation, each population has a potential for seed production of 314,554 seeds (Ile Mandiri) and 529,538 seeds (Ile Boleng) every year.

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Sudirja R · Rosniawaty S · Rahayu SM · Qurrohman BFT

Effectiveness of modified nitrogen fertilizer on soil chemical properties and rice plant growth in the textile industrial area

Abstract. The imbalance of nutrients and heavy metal contamination is a challenge in increasing plant growth surround the textile industry area. This study aimed to determine the effectiveness of the dosage of Biologically Agent N Organo Mineral Fertilizer (Biomix-N) as a fertilizer and an ameliorant in paddy soil contaminated with textile liquid waste. This study used a randomized block design of eight treatments with four replications. Parameters observed were soil chemical properties after application of Biomix-N (Na-ex, total N, EC, Cd, and pH), rice plant growth (plant height and tiller number), Cd concentration in the plant, and relative agronomic effectiveness (RAE). Data analysis used analysis of variance, Duncan's test at a 5% level, regression, and correlation analysis. The results showed that Biomix-N 500 kg ha⁻¹ affected the value of EC and total N, while it did not affect Na-ex, Cd, pH and Cd uptake by the paddy plant. Biomix-N fertilization affects the height and number of tillers of rice plants at the age of 70 DAP. Biomix-N fertilization 500 kg ha⁻¹ gave an RAE value of 126-176% compared to the control treatment. The total N content of the soil has a high value of coefficient determinant and correlation ($R^2= 0.76$; $R = 0.9$) on the growth of rice plants. Applying Biomix-N 500 kg ha⁻¹ equal to 300 kg ha⁻¹ of urea was an effective dose for paddy soil surround the textile industrial area.

Keywords: Cadmium · Nutrient balance · Soil amendment

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Sudirja R^{1*} · Rosniawaty S² · Rahayu SM³ · Qurrohman BFT⁴

¹ Department of Soil Science and Land Resources Management, Faculty of Agriculture, Universitas Padjadjaran – Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia

² Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran –Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia

³ Study Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran –Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia

⁴ Doctoral Program of Agricultural Sciences, Faculty of Agriculture, Universitas Padjadjaran –Jl. Raya Bandung Sumedang Km. 21 Sumedang 45363, Indonesia

*Correspondence: rija.sudirja@unpad.ac.id

Introduction

Rice is a staple food for the majority of the population in Indonesia. The increase in production is directly proportional to the expansion of the land area for growing rice and increasing the productivity of rice plants (Qurrohman et al., 2023). However, there are challenges in increasing the land area for rice cultivation due to the need for converting paddy fields for other purposes, such as infrastructure, residential, and industrial development. This conversion negatively impacts the available land area and the ecological conditions in the surrounding areas.

According to Afrad et al. (2020), converting paddy fields into industrial areas, particularly for the textile industry, leads to a decline in soil and water quality, as well as the quality and quantity of rice yields. Hossain et al. (2015) found that paddy field soils irrigated with textile waste-contaminated water exhibited increased levels of several elements such as Ca, Mg, Fe, Mn, Cu, and Na, as well as heavy metals like Pb, Cd, Ni, and Cr. The accumulation of these elements in the soil leads to an imbalance in nutrient availability (Parent et al., 2016), increased salinity, and accumulated heavy metals, intensifying abiotic stress in rice plants (Imtiaz et al., 2016).

Efforts to enhance rice productivity on soil polluted by textile waste faced the challenge of addressing nutrient imbalances, reducing heavy metal concentrations, and decreasing salinity.. One of the alternative approaches to tackle these issues is by modifying fertilizers and soil ameliorants (Sudirja et al., 2019).

One approach to remediate polluted paddy fields is by reducing the availability of heavy metals and sodium. Heavy metal in the soil affected N uptake by the plant (Blaudez et al., 2000). An alternative is to use N Organo Bio-agent Mineral (Biomix-N) modified nitrogen fertilizer, which consists of urea with ameliorant ingredients such as zeolite, activated charcoal, and bio-agent compost. Zeolite and activated charcoal act as adsorbents for contaminants, like the heavy metal Cd, and can reduce salinity (Ghasemi et al., 2017; Dosa et al., 2022). In addition, compost enriched with the biological agent *Bacillus subtilis*. Syed and Chinthala, 2015 reported that *Bacillus subtilis* had a maximum biosorption of heavy metal. The compost was

increase organic matter in the soil and also contributes to the reclamation of polluted land by binding the heavy metals with the negative charge of functional groups of humus substances (Wu et al., 2017; Masykuri and Setyono, 2019). The Biomix-N is formulated to fulfill N nutrient for paddy plant and in the same time decrease heavy metal toxicity.

This study aimed to determine the effectiveness of the dosage of Biologically Agent N Organo Mineral Fertilizer (Biomix-N) as a fertilizer and an ameliorant in paddy soil contaminated with textile liquid waste.

Materials and Methods

The research was carried out in paddy fields (Table 1) in Rancaekek District, Bandung Regency, West Java, with an altitude of ± 676 m asl ($6^{\circ} 58' 10.6''$ S; $107^{\circ} 46' 58.7''$ E). Fertilizer production and soil plant chemical analysis were done at the Soil Fertility and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran. This research was conducted from September to November 2019.

Table 1. Paddy soil chemical properties.

	Soil Properties	Unit	Values	Category *
1	pH H ₂ O	-	6.72	Neutral
2	pH KCl	-	4.98	-
3	C-organic	%	1.5	Low
4	Total-N	%	0.84	High
5	NH ₄	%	0.16	Low
6	NO ₃	%	0.21	Medium
7	Pb	Ppm	21.66	Low
8	Cd	Ppm	0.04	Low
9	Cr	Ppm	75.44	Polluted
10	Na-ex	%	9.46	Medium
11	Electrical conductivity (EC)	dS/m	1.06	Low
	Cation exchange capacity (CEC)	cmol(+) / kg	43.32	Very high
12		g		

*Criteria based on Indonesia Soil Research Institute (2009)

The materials used in this study: rice seeds of the Inpara 9 Agritan variety, urea, activated charcoal, *Bacillus subtilis* enriched compost, zeolite, basic fertilizer SP-36 (1.12 g plant⁻¹) and

KCl (0.56 g plant⁻¹). The formulation used in the manufacture of N Organo Mineral Fertilizer with Biological Agents was urea, zeolite, activated charcoal, compost enriched with *Bacillus subtilis* with ratio 60:20:10:10, respectively. The results of laboratory analysis indicated that the N Organo Mineral Fertilizer (Biomix-N) contained 22% nitrogen, 0.03% P₂O₅, and 0.3% K₂O.

The equipment used in this study were: field equipment (hoes, tape measure, plastic rope, stakes, scissors, shovels, hoes, paper, labels, ticks, manual weeder, plastic samples, rulers and stationery), equipment for making N Organo Mineral fertilizers (granulators, sieves, analytical balances, buckets, stirrers, zip plastic) and laboratory equipment for soil and plant analysis.

The experimental design was a randomized block design (RBD) consisting of eight treatments, and each was replicated four times. The study was examined six-level doses of N organo Mineral fertilizer (Biomix-N), 100% urea treatment, and control (without fertilizer) (Table 2). This present study used a dose of Biomix-N between 250-1500 kg ha⁻¹ to determine the effectiveness of low to high doses of Biomix-N.

Parameters assessed included pH, Total-N, electrical conductivity (EC), Na-ex, Cd in the soil, relative agronomic effectiveness (RAE), Cd concentrations in the plant, plant height and tillers at 70 days after transplanting (DAP).

Table 2. Application of N organo mineral fertilizer (Biomix-N).

Symbol	Treatment	Doses of fertilizer (kg ha ⁻¹)
A	Control Negative	0
B	Control Positive (Urea)	250
C	Biomix-N	250
D	Biomix-N	500
E	Biomix-N	750
F	Biomix-N	1000
G	Biomix-N	1250
H	Biomix-N	1500

This research was divided in two steps (Figure 1). The first step was soil sampling, in which the paddy soil from textile industrial area were collected to be analyzed. The second step

involved the cultivation of paddy plant. The Biomix-N was applied directly in to the soil seven days before the paddy plant was transplanted.

To assess the impacts of the treatments on pH, electrical conductivity, N-total (Kjeldahl method), Na-ex (Flame photometry), Cd, Cd absorption, plant height and number of tillers, data analysis was conducted using analysis of variance (ANOVA) at α= 5% significance level and Duncan's multiple range test at α= 5%. Pearson correlation analysis was done to examine the relationship between pH, electrical conductivity, exchangeable sodium (Na-ex), total N, and the variables of plant height and tillering. The RAE value was calculated using the following equation (Mackay et al., 1984):

$$RAE = \frac{X_i - C}{X_c - C} \times 100\%$$

Where:

RAE= Relative Agronomic Effectiveness

X_i = Plant growth fertilized with Biomix-N

X_c = Plant growth fertilized with Urea

C= Control



Figure 1. Research stage procedure: (A) soil survey, (B) transplanting paddy plant and (C) measuring plants growth parameter.

Results and Discussion

The effect of Biomix-N fertilizer on the soil pH. The analysis of variance showed that Biomix-N had no effect on the pH of paddy soil ($P > 0.05$). However, when Biomix-N fertilization was applied at doses ranging from 250-1500 kg ha⁻¹ or the equivalent of 150-900 kg ha⁻¹ urea, along with the inclusion of zeolite and organic matter, it was observed to reduce the adverse impact of urea on soil pH (Figure 2). According to Tong & Xu (2012), the application of urea fertilizer alone can accelerate the decrease in soil pH.

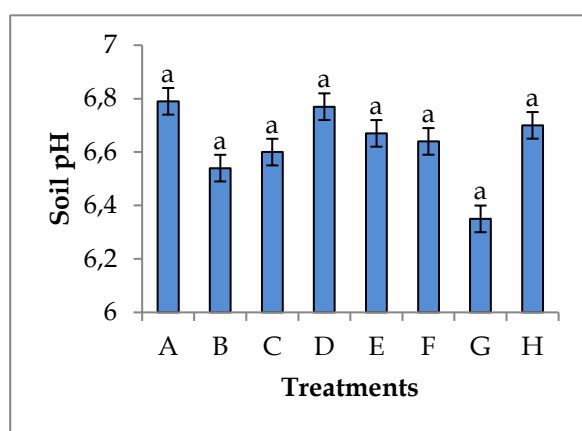


Figure 2. Effect of Biomix-N fertilizer on the soil pH.

The effect of Biomix-N fertilizer on the total N. The results showed that the application of Biomix-N affected the N content in each treatment (Figure 3). The dose of Biomix-N 500 kg ha⁻¹ (Treatment D) was significantly different compared to the control without N (A) fertilization. Fertilization with 500 kg ha⁻¹ Biomix-N, or equivalent to 300 kg ha⁻¹ of urea fertilizer, was not significantly different from the treatment of 250 kg ha⁻¹ (B) urea fertilizer, 250 kg ha⁻¹ Biomix-N and 750-1500 kg ha⁻¹ Biomix-N. The use of Biomix-N fertilizer could save up to 40% of the use of urea fertilizer on rice plant growth. According to He et al. (2002), applying zeolite and compost can increase the N availability in sandy soils. The outcome of this study provides additional information, highlighting that the role of zeolite and compost on clay is similar in enhancing the efficiency of N fertilization. In addition, Aslam et al. (2021) reported that the addition of zeolite and biochar can increase soil nutrient availability.

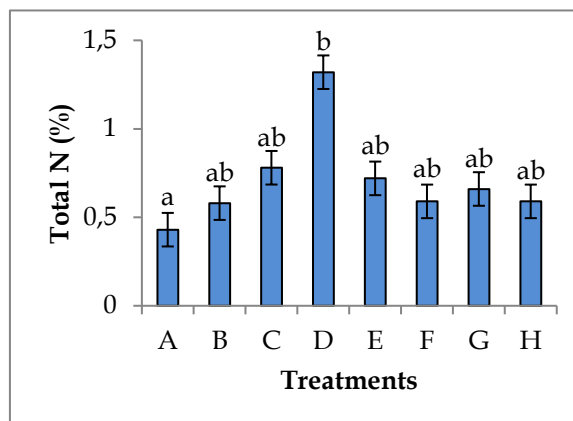


Figure 3. Effect of Biomix-N fertilizer on the soil total N.

Effect of Biomix-N fertilizer on the soil electrical conductivity (EC). The analysis of variance showed that the application of different doses of Biomix-N affected the EC of the soil solution (Figure 4). The dosage of Biomix-N fertilizer 250-1500 kg ha⁻¹ was similar compared to the control (urea fertilizer 250 kg ha⁻¹) and without fertilization. The initial chemical analysis (Table 1) of paddy soil in this study showed an EC value of 1.06 dS m⁻¹. This value does not classify the soil as saline soil (Osman, 2013), as soil is considered saline when the EC value exceeds 4 dS m⁻¹. The soil analysis conducted at the end of the vegetative phase demonstrated that applying Biomix-N at doses ranging from 250-1500 kg ha⁻¹ was not significantly different compared to the control and without fertilization. These results indicated that fertilization with Biomix-N did not cause salinity in paddy fields.

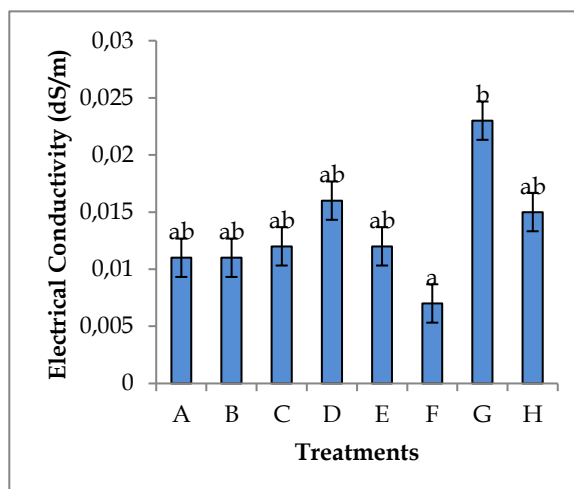


Figure 4. Effect of Biomix-N fertilizer on the soil electrical conductivity.

The effect of Biomix-N fertilizer on exchangeable soil sodium. The application of Biomix-N fertilization did not show a significant effect on Na-ex (Figure 5). The decrease in Na-ex was not significantly different in the C-H treatment compared to the control (A). The contents of zeolite and compost in Biomix-N did not reduce the Na-ex content significantly. According to Ghorbani et al. (2022), zeolite contains Na^+ , which can increase the Na-ex in the soil. The role of compost in reducing Na-ex can only partially solve the problem of soil salinity, but adding compost will improve soil physical properties and other chemical properties (Lakhdar et al., 2009).

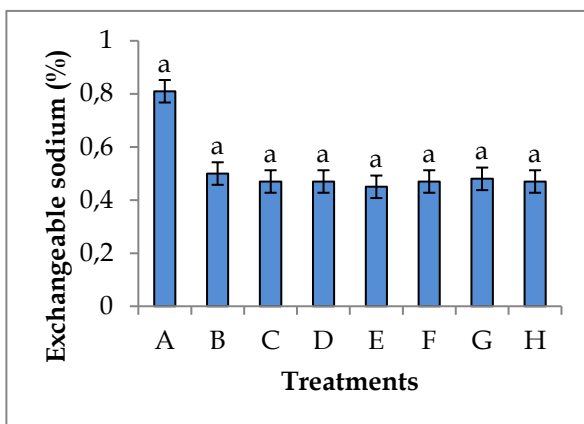


Figure 5. Effect of Biomix-N fertilizer on the soil exchangeable sodium (Na-ex)

The effect of Biomix-N fertilizer on Cd concentration in the soil. Based on the data shown in Table 3, the total Cd content in paddy fields after the treatment was not detected or the amount was lower than 0.01 ppm. This showed that total Cd in the soil decreased compared to initial soil analysis (0.04 ppm).

Table 3 Concentration of Cd in the soil after treatments.

Treatments		Total Cd (ppm)
A	Control Negative	nd ^{a)}
B	Control Positive (Urea)	nd ^{a)}
C	Biomix-N 250 kg ha ⁻¹	nd ^{a)}
D	Biomix-N 500 kg ha ⁻¹	nd ^{a)}
E	Biomix-N 750 kg ha ⁻¹	nd ^{a)}
F	Biomix-N 1000 kg ha ⁻¹	nd ^{a)}
G	Biomix-N 1250 kg ha ⁻¹	nd ^{a)}
H	Biomix-N 1500 kg ha ⁻¹	nd ^{a)}

Remarks: nd^{a)}: concentration Cd can not be measured because lower than 0.01 ppm

An increased pH value causes a higher metal ion adsorption due to competition with H^+ ions during cation exchange. In addition, the Biomix-N fertilizer contains zeolite and activated charcoal which have a high adsorption of metal ions (Guo et al., 2021).

The effect of Biomix-N fertilizer on Cd absorption by the paddy plant. The high mobility characteristic of Cadmium in the soil easily absorbed by plants. Rice plants accumulated Cd higher than other types of cereals when planted in Soil containing Cd (Gao et al., 2016). Based on the data showed in Table 4, Cd uptake in rice plants after treatment was not detected or the amount was smaller than 0.01 ppm. The concentration of Cd in the soil before treatment and after treatment was still below the Cd tmaximum value of 0.4 ppm (FAO, 2001).

Table 4 Concentration of Cd uptake by paddy plant.

Treatments		Total Cd (ppm)
A	Control Negative	nd ^{a)}
B	Control Positive (Urea)	nd ^{a)}
C	Biomix-N 250 kg ha ⁻¹	nd ^{a)}
D	Biomix-N 500 kg ha ⁻¹	nd ^{a)}
E	Biomix-N 750 kg ha ⁻¹	nd ^{a)}
F	Biomix-N 1000 kg ha ⁻¹	nd ^{a)}
G	Biomix-N 1250 kg ha ⁻¹	nd ^{a)}
H	Biomix-N 1500 kg ha ⁻¹	nd ^{a)}

Remarks: nd^{a)}: concentration Cd can not be measured because lower than 0.01 ppm

Paddy Plant Growth (Plant Height and Number of Tillering). Based on Figure 6-7, the application of Biomix-N fertilizer affects the plant height and the number of tillers. The Biomix-N fertilization at a dose of 500 and 750 kg ha⁻¹ showed a significant difference compared to the treatment without urea fertilization (control negative) on plant height at 70 DAP.

The results also showed there was non significant difference between the application of urea fertilization 250 kg ha⁻¹ (B) and Biomix-N fertilizer at doses ranging from 250-1500 kg ha⁻¹. These findings indicated that Biomix-N fertilization can effectively sustain plant growth in conditions of excessive urea fertilization, thereby serving as a soil amendment (Guo et al.,

2021). The increased of rice plants growth was obtained with Biomix-N 250-500 kg ha⁻¹.

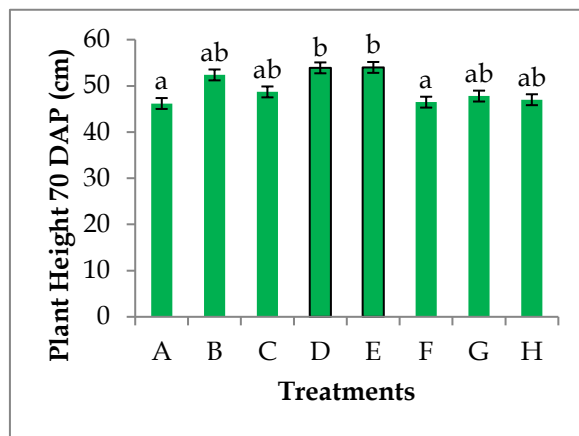


Figure 6. The effect of Biomix-N on paddy plant height 70 days after transplanting (DAP).

Based on figure 6 and 7 Biomix-N 500 kg ha⁻¹ was reached the optimum paddy plant height and tillering at 70 DAP. Biomix-N 500 kg ha⁻¹ was equivalent to 300 kg ha⁻¹ of urea fertilizer. Wang et al. (2017) reported that increase N level fertilizer improved plant growth. Nitrogen as a macro element was needed by plant in the large amount.

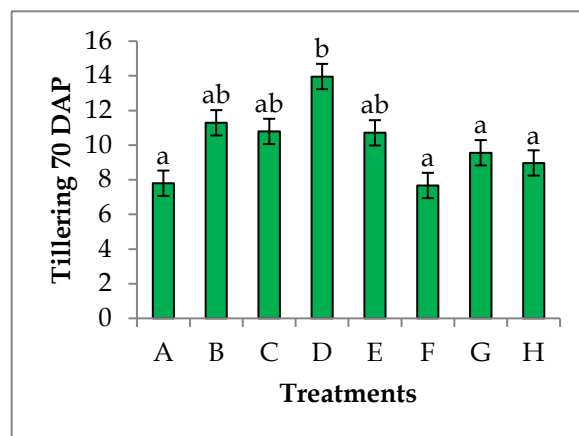


Figure 7 The effect of Biomix-N on paddy plant tillering 70 days after transplanting (DAP).

Based on the Relative Agronomic Effectiveness value (Table 3), the highest percentage of rice plant growth was obtained using 500 kg ha⁻¹ Biomix-N fertilization.

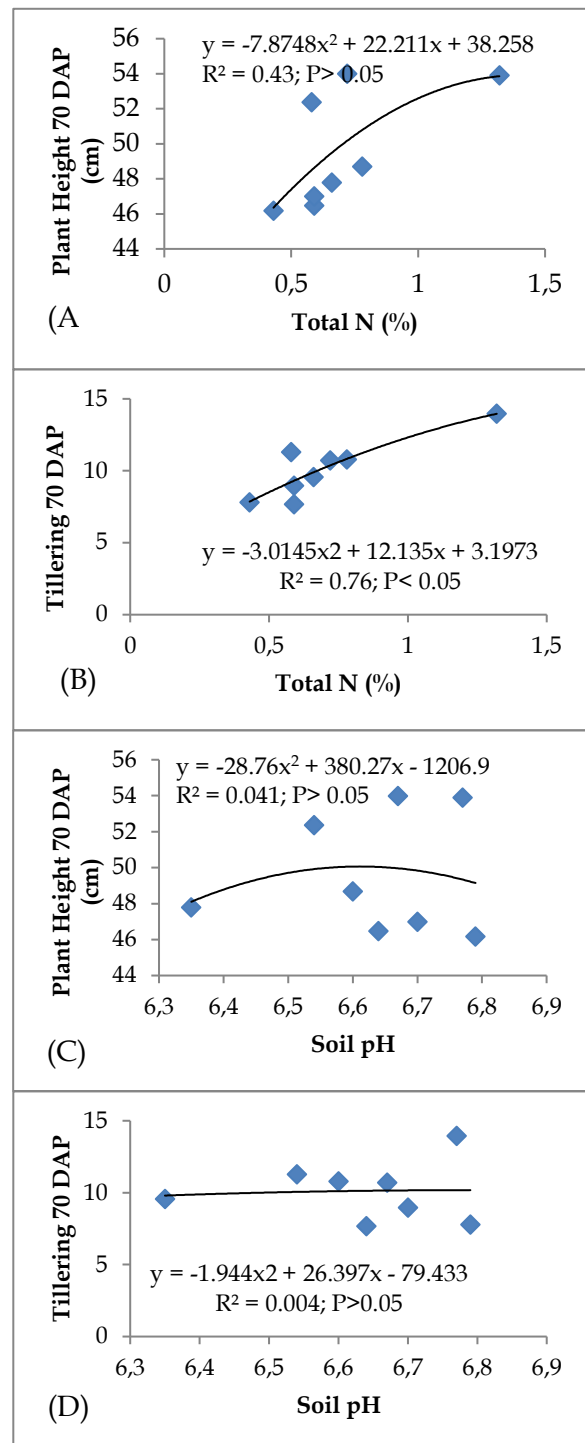


Figure 8 Correlation between total N (A, B) and soil pH (C, D) with plant height and tillering paddy plant.

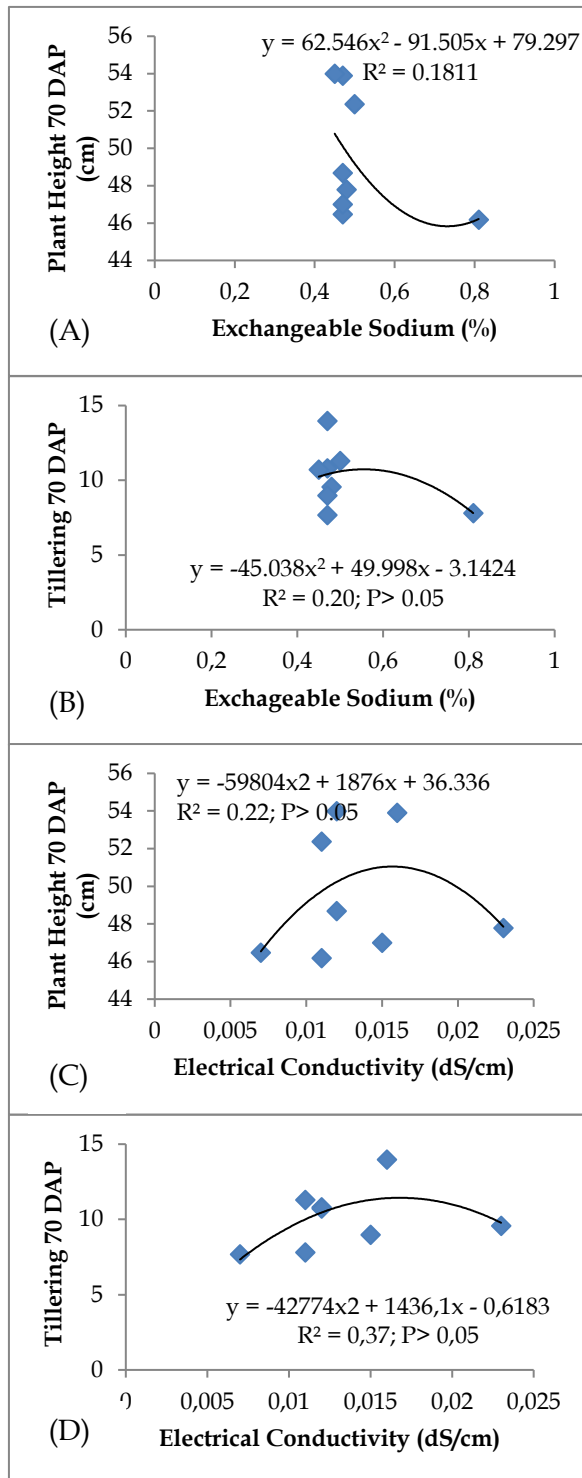


Figure 9. Correlation between Na-ex (A, B) and soil EC (C, D) with plant height and tillering paddy plant.

Table 3. Relative Agronomic Effectiveness (RAE).

	Treatments (kg ha ⁻¹)	RAE (%)	
		Plant height (cm)	Tillering
A	Control negative	-	-
B	Control positive (Urea)	-	-
C	Biomix-N 250	40.55	85.67
D	Biomix-N 500	124.72 [†]	176.50 [†]
E	Biomix-N 750	126.17 [†]	83.38
F	Biomix-N 1000	4.68	3.72
G	Biomix-N 1250	26.01	50.43
H	Biomix-N 1500	13.25	33.52

Remark: [†] RAE > 100%

Relationship between Soil Chemical Properties on Plant Height and Number of Tillers. Based on Fig. 8-9 (A, B, C and D), the highest coefficient of determination between Na-ex, N-total, pH and EC with the height and number of tillers of rice plants at 70 days after planting (DAP) was obtained at the N-total, with the plant height ($R^2 = 0.43$) and a number of tillering ($R^2 = 0.75$). The coefficients of determination for Na-ex, EC and soil pH were relatively small. However, Na-ex exhibited a negative correlation coefficient, indicating that an increase in soil Na-ex can reduce the growth of rice plants (Putra et al., 2021).

Conclusion

Application of Biomix-N 500 kg ha⁻¹ affected the soil EC and total N. However, it did not affect exchangeable sodium, Cd soil pH and Cd uptake by paddy plant. The Biomix-N 500 kg ha⁻¹ fertilization also influenced the height and the number of tillers of rice plants at the age of 70 DAP. Specifically, the application of Biomix-N fertilization at a rate of 500 kg ha⁻¹ resulted in a RAE value ranging from 126-176% compared to the control treatment. The total N content of the soil had a high correlation value ($R = 0.9$) with the growth of rice plants. Therefore, applying Biomix-N 500 kg ha⁻¹ which is equivalent to 300 kg ha⁻¹ of urea was an effective dose for paddy soil in the industrial area.

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Suwitono B · Chozin MA · Guntoro D · Suwarto

Response of four local cassava accessions to bio-mulch application time

Abstract. Cassava is widely planted on marginal land with low soil fertility. During the initial growth phase, cassava often loses competition against weeds. Legume cover crops are widely used to increase soil fertility, prevent erosion, and suppress weeds. This study aimed to determine the effect of *Arachis pinto* bio-mulch application time on the growth and yield of cassava. This study was conducted using a factorial randomized block design. The first factor was four cassava accessions: Ketan Malang, Genjah Bayam, IR Jonggol, and Mangu. The second factor was six levels of bio-mulch planting time: manual weeding (without bio-mulch applications), bio-mulch planting at the same time as cassava planting, and four, eight, and twelve weeks before cassava planting. The observations included plant height, stem diameter, number of tubers, tuber weight, tuber length, plant biomass, dry matter, and productivity. The results showed that all cassava accessions responded similarly to the planting time of *A. pinto* bio-mulch. Different bio-mulch application time was insignificant in the cassava growth, except for the number of tubers and tuber diameter. The twelve weeks before cassava planting tends to reduce the results of cassava accessions.

Keywords: *Arachis pinto* · Cover crop · Dry land · Growth · Yield

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Suwitono B^{1,3*} · Chozin MA² · Guntoro D² · Suwarto²

¹ Departement Agronomy and Horticulture Graduate School, Faculty of Agriculture, IPB University.
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia.

² Departement Agronomy and Horticulture, Faculty of Agriculture, IPB University.
Jl. Meranti Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia.

³ Food Crops Research Center, National Research and Innovation Agency, Cibinong Science Center,
Jl. Raya Jakarta- Bogor, Cibinong, Bogor 16915, West Java, Indonesia

*Correspondence: bayu030@brin.go.id

Introduction

Cassava is one of the promising commodities in the agricultural sector as a multifunctional crop that can be utilized as a raw material for alternative energy sources, food, and feed (Kristian, 2015). In 2020, Indonesia's total cassava production reached 18 million tons from a harvested area of 701,000 hectares. In 2017-2020, fluctuations emerged in cassava production in Indonesia, as the total cassava production decreased to 16 million tons in 2018, then increased in the following year (FAO, 2020). During 1970-2017, cassava production in Indonesia increased by 19 million tons, below Nigeria at 49 million tons and Thailand at 31 million tons (Ikuemonisan et al., 2020).

Cassava can become a solution for marginal and critical land use (Saidi, 2020). Mostly, cassava commodities are grown by farmers on marginal lands with less optimal soil based on the physical, chemical, and mineral properties. Furthermore, cassava is mainly developed on alfisol, ultisol, entisol, and inceptisol soil types with low nutrient status, low organic matter, and highly vulnerable to erosion. According to Radjit et al., (2008), cassava development in the following lands could show significant growth and increased production. Moreover, proper cassava varieties are also necessary for efficient cultivation technology.

However, the development of cassava is relatively long, e.g., 8-12 months. Also, cassava has a relatively low selling price, decreased nutrient contents, when transported to the market, and an inability to protect the soil from rainwater, thus vulnerable to erosion (Pramudita et al., 2014).

Cassava production is also affected by weeds in a rapid cassava cultivation system. Weeds are competitors for cassava plants that can reduce cassava yields by 75% in the first three months of growth. Similarly, Suwanto (2012) stated that the initial growth phase is one of the weaknesses in cassava planting, as it cannot compete with weeds at a critical period of 5-10 weeks after planting. The broadleaf weeds from the Asteraceae family are found around one-month-old cassava plantations. In addition to inhibiting cassava growth, weeds are a gathering place for cassava pests (Putra and Jeclin, 2019).

An ornamental bean, *Arachis pintoi*, is a bio-mulch or a living mulch that can grow anywhere easily. *A. pintoi* can bind nitrogen from the air,

which can be applied as a land cover plant, an ornamental plant in city parks, and erosion control on sloping land (Maswar, 2004). According to Insani et al. (2020), *A. pintoi* is a legume that can be planted using a mixed cropping pattern. Various cropping patterns between corn and *A. pintoi* can increase corn production.

According to Suwanto and Asih (2021), using LCC and organic matter elevation can also increase the soil moisture. Badriyah and Chozin (2017), stated that planting the *A. pintoi* beans as bio-mulch on sweet corn plants with minimal tillage can increase the sweet corn production. Meanwhile, Zhong et al. (2018) stated that long-term use of *A. pintoi* and *C. rotundifolia* in peach plantations improved the soil chemical properties and increased the soil organic matter. Furthermore, Evizal (2003), described that *A. pintoi* had several advantages, including drought resistance, shade tolerance, high biomass production capability, and low propagation on the mother plant.

This study aimed to evaluate the benefits of *A. pintoi* as a bio-mulch with different cropping times in cassava cultivation, expected to increase cassava fertility and yield.

Materials and Methods

This study was conducted from November 2021 – September 2022 in the IPB Teaching Farm, Faculty of Agriculture, IPB University. This area is located at 6°28'12" South Latitude and 107°01'47" East Longitude. The soil type at the study site was ultisol soil, dominated by clay texture with low soil pH < 5. Rainfall during the study was observed from the average number of rainy days (Figure 1).

The materials used in this study were *A. pintoi*, four accessions of cassava plants, i.e. *Ketan Malang*, *Genjah Bayam*, *IR Jonggol*, and *Mangu*, cow manure, urea, KCL, SP-36, glyphosate herbicides, and carbofuran pesticides. The study used a two-factor factorial randomized block design (RBD). The first factor was the cassava variety types (V), namely *Ketan Malang* (V1), *Genjah Bayam* (V2), *IR Jonggol* (V3), and *Mangu* (V4). The second factor was the planting time levels of *A. pintoi* bio-mulch, namely manual weeding (without *A. pintoi* bio-mulch applications) as a control (B0), 0 week of planting bio-mulch, before cassava planting (B1), planting

bio-mulch on four weeks before cassava planting (B2), planting bio-mulch on eight weeks before cassava planting (B3), and planting bio-mulch on 12 weeks before cassava planting (B4).

The cultivation land was prepared manually, and the experimental plots were made with a 5 m x 5 m size and 100 cm distance between plots. The soil was processed to 20 cm depth, raked and leveled with a hoe to form beds at 5 m x 1 m. For *A. pinto*, stem cuttings from the following plants were propagated by themselves. The cutting size was uniform at 12 cm length. *A. pinto* cuttings were planted in polybags at 10 cm x 15 cm, one cutting in one polybag with soil and husk mixtures (2:1). After five weeks, the *A. pinto* cuttings were transferred to the plot, according to the treatment at planting with 20 cm distance between plants.

For *A. pinto* treatment applications, cassava plants were planted simultaneously after *A. pinto* planting at 0 week after planting (WAP). Cassava cuttings were obtained from the cassava stems at 8-12 months with cuttings 25 cm long. Cuttings were planted upright, i.e., 2/3 in the ground. The spacing used 100 cm x 100 cm, where the distance between rows was 100 cm, with one cutting in each hole. There were 25 cassava plants with five sample plants in one experimental plot.

Fertilization with manure was applied by planting the cassava at 5 tons/ha. Fertilization with chemical properties was applied manually about 15 cm from the cassava plant, whereas 300kg.ha⁻¹ urea, 100kg.ha⁻¹ SP36, and 100kg.ha⁻¹ KCl was performed twice, namely, urea was applied with 1/3 dose of all fertilizers. Meanwhile, the SP36 and KCl were applied ten days after planting, whereas 1/3 of urea was applied three months after planting. For the rest five months, urea was applied directly after planting.

The observed growth and yield variables included plant height (cm), stem diameter (mm), and plant yield components. The plant yield components were composed of plant biomass weight, number of tubers per plant, tuber diameter (mm), tuber length (cm), wet tuber weight per plant (kg), productivity (t/ha), and tuber dry matter (dry matter). Growth and yield data were obtained when the plants reached six months old or at harvest.

All data were analyzed with an analysis of variance at the 5% significance level using SPSS 22.0 to determine differences in the effect of the average treatment. If a significant different value

was obtained among the data, the test was continued with an honest significant difference test (Tukey's test) at a significant level of 5% to determine the highest treatment effect among the treatments.

Results and Discussion

Components of cassava growth. The variance results showed no interaction between cassava accession and application time of *A. pinto* bio-mulch on plant height and stem diameter. However, the height and stem diameter increased along with the increasing plant age. Cassava accession treatment and bio-mulch application time significantly affected the plant height and stem diameter (Table 1). Total plant height ($p < 0.05$) was affected by the cassava accessions. The plant height of *IR Jonggol* cassava was higher than *Ketan Malang*, *Genjah bayam*, and *Mangu* during the vegetative growth until the harvesting age of 6 MAP (months after planting). The *IR Jonggol* accession had a genotype characterized by upright and symmetrical growth without branching. Moreover, the *IR Jonggol* are cassava types that are cultivated on ultisol soil conditions in the Jonggol area, thus more adaptive in their vegetative growth. According to Diaguna et al. (2022), plant height is one of the well-adapted morphological characteristics of plants based on the shape of the lobes and petioles. At 12 weeks before planting the cassava application, the bio-mulch application showed the lowest plant height compared to other application times. The growth of *A. pinto* bio-mulch at 12 WBP may be highly fertile, affecting the cassava's vegetative growth. In addition, high rainfall (figure 1) in December accelerated the growth of *A. pinto* bio-mulch.

The accession types affect cassava stem diameter. The *IR Jonggol* cassava stem diameter was larger than other cassava accessions. This condition indicates a relationship between plant height and stem diameter. The taller plant height, the wider stem diameter. Meanwhile, the 12 WBP bio-mulch treatment showed a smaller stem diameter than other bio-mulch application treatments. The low diameter of cassava stems impacted the number of roots and tubers. According to Suwitonon et al. (2017), the size of the stem diameter affects the weight of the tubers and the number of cassava tubers. Cassava plant height significantly differed, whereas the *IR Jonggol* accession could grow well on Jonggol

ultisol soil. In contrast to *Mangu* and *Genjah Bayam*, both accessions had lower plant heights than others. According to Cock and Connor (2021), cassava can grow well in various soil types that are low in nutrients. In common nutrient conditions, the crown growth is reduced, and tuber growth is increased. The plant height of *IR Jonggol* cassava in this study reached 3.11 m, which can be helpful as a source of cutting material for the next growing season. According to Misganaw and Bayou (2020), varieties and genetics affect the high and low of cassava plants. A taller plant can be used as a cutting material for the next growing season.

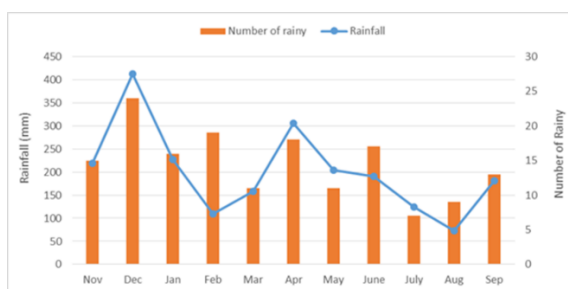


Figure 1. Rainfall data at the study site on November 2021 – September 2022. The line graphs show the rainfall and the bar graphs show the number of rainy. (Source: BMKG Bogor, West Java)

Tuber yield and yield components of cassava accessions. The number of tubers per plant is strongly influenced by the application time of the bio-mulch (Table 2). The 12 WBP (weeks before planting) cassava with bio-mulch application treatment produced the least number of tubers per plant, compared to other bio-mulch applications. According to Siswati et al. (2019), cassava tubers began to form from developing nodal and basal roots on a month after planting,. Soil as a medium for plant growth and soil conditions can affect the plant root system in the tuber formation. The plant root system includes the availability of water, nutrients, and aeration, which also involves the root spreading. With the bio-mulch application treatment, it is important to determine the bio-mulch spacing, so no competition between cassava and bio-mulch will occur.

The tuber length was significantly affected by the cassava accession. The tuber length of *Genjah Bayam* and *Mangu* accession exceeds the *Ketan Malang* and *IR Jonggol* (Table 2). This is possible, because *Genjah Bayam* and *Mangu*

accessions have long tuber genetical characteristics. According to Listyowati and Sutarno (2017), the length and shape of the tubers are influenced by genetic factors, cropping systems, and fertilization. In addition, the greater the number of tubers produced as sink organs, the more competition between sinks (tubers) can occur, which affects the length of tubers.

The type of cassava accession influenced the actual tuber diameter. Cassava accession of *Ketan Malang* produced the largest tuber diameter, compared to *Genjah Bayam*, *IR Jonggol*, and *Mangu*. These results indicate that the *Ketan Malang* accession with many twigs and double branches will produce more harvesting product, thereby increasing the assimilated products, that will be transported to sink organs, including tubers. In addition, the *Ketan Malang* cassava is quite adaptive to ultisols with a soil pH of <5. More tubers will likely be marketed, if the tuber diameter is more than 50 mm.

Meanwhile, the bio-mulch application time treatment showed that the bio-mulch treatment time on 12 weeks before cassava planting increased the cassava yield with the largest tuber diameter of 42.68 mm. It is possible that the presence of bio-mulch from the legume cover crop can reduce the soil bulk density. Soil conditions with low bulk density triggers the cassava tubers to grow and develop. Soil tillage, use of cover crops, and crop rotation are some of the soil conservation practices that can affect the soil bulk density (Abrougui et al., 2014). According to Nascente et al., (2015), the use of cover crops significantly affects the soil density and increases the organic C content in the soil layer.

Tuber weight per plant was significantly affected by the treatment time, which was significantly affected by the bio-mulch application time. The four applications of bio-mulch had no significant difference, except the 12 MAP bio-mulch treatment, which caused the lowest tuber weight per plant of 2.91 kg (Table 2). This was possible due to the relationship between the number of tubers and the weight of tubers per plant. Because the number of tubers in the B4 treatment was less, the weight of tubers per plant became smaller. The correlation value between the tuber weight per plant and the number of tubers per plant was ($r = 0.72^{**}$) (Table 4), which indicates a positive correlation between both parameters. According to Hamdani et al., (2021), high tuber weight comes from the amount of

assimilated product from plants. According Kaluba et al. (2022), planting cassava and legumes can increase the soil organic matter, besides producing the yield loss, which must be insignificant. The low yield of tubers from *Mangu* cassava and *Genjah Bayam* was possible because the leaf stalks and shapes of both plants were longer and wider, so they shaded each other between plants. The influence of shade between plants will receive sunlight less optimal. Therefore, *Genjah Bayam* and *Mangu* planting should consider the ideal spacing of more than one meter. According to Adeniji et al., (2011), the physiological process of the plant is still running at six months, maximally decreasing the tuber yield of 12 months after planting.

The weight of plant biomass at the harvesting stage, after six months after planting (MAP) was influenced by the interaction between cassava accession and the bio-mulch application time. The *IR Jonggol* accession without the bio-mulch obtained the highest biomass weight at 3.42 kg (Table 3). This indicates that the *IR Jonggol* accession, with growing upright, strong and tall stem characteristics, produces more biomass in the shoots. In contrast to *Mangu* accession with B4 treatment, the lowest biomass was at 2.13 kg. This condition presents that vegetative growth, especially plant height from *Mangu* accession, was lower, resulting in a lower plant biomass weight. According to de Souza et al. (2017), the total plant biomass is produced from overall photosynthesis assimilation.

Table 1. Plant Height (m) and stem diameter (mm)

Treatment	Plant Height (m)				Stem Diameter (mm)			
	3 MAP	4 MAP	5 MAP	6 MAP	3 MAP	4 MAP	5 MAP	6 MAP
Accession								
<i>Ketan Malang</i>	1.45 b	1.98 b	2.40 b	2.60 b	21.85 b	24.31 b	25.35 b	26.34 b
<i>Genjah Bayam</i>	1.21 c	1.68 c	2.11 c	2.36 c	22.73 b	25.87 ab	26.95 ab	27.75 ab
<i>IR Jonggol</i>	1.90 a	2.46 a	2.90 a	3.11 a	24.52 a	27.24 a	27.81 a	28.27 a
<i>Mangu</i>	1.23 c	1.68 c	2.13 c	2.35 c	22.73 b	25.15 b	26.63 ab	27.33 ab
Bio-mulch								
B0	1.58 a	2.08 a	2.53 a	2.72 a	24.15 a	26.96 a	27.63 a	28.56 a
B1	1.55 a	2.02 ab	2.44 a	2.63 ab	23.93 a	26.21 a	27.05 ab	27.45 ab
B2	1.51 ab	2.03 ab	2.45 a	2.64 ab	23.53 a	26.38 a	27.01 ab	27.85 ab
B3	1.36 bc	1.91 b	2.38ab	2.61 ab	22.42 ab	25.13 ab	26.48 ab	27.32 ab
B4	1.23 c	1.72 c	2.16 b	2.42 b	21.04 b	23.53 b	25.24 b	25.91 b

Note: Means followed by the same letters in the same row are insignificantly different according to Tukey test $\alpha=5\%$. B0: without bio-mulch (control), B1: 0 week before cassava planting, B2: 4 weeks before cassava planting, B3: 8 weeks before cassava planting, B4: 12 weeks before cassava planting.

Table 2. The effect of accession type and bio-mulch application time on the yield component of cassava at six months old

Treatment	Number of tubers/plant	Length of tubers (cm)	Diameter of tubers (mm)	Fresh tubers weight/plant (kg)
Accession				
<i>Ketan Malang</i>	15.1	36.35 b	50.15 a	3.62
<i>Genjah bayam</i>	14.8	41.70 a	44.94 b	3.53
<i>IR Jonggol</i>	16.7	35.90 b	46.03 b	3.45
<i>Mangu</i>	14.1	41.05 a	44.53 b	3.13
Bio-mulch				
B0	16.1 a	41.18	46.62 ab	3.68 a
B1	17.6 a	37.12	44.54 b	3.60 a
B2	16.1 a	39.25	46.10 ab	3.55 a
B3	14.1 ab	38.81	47.12 ab	3.41 a
B4	11.7 b	37.37	47.68 a	2.91 b

Note: Means followed by the same lowercase alphabet in the same column is insignificantly different based on Tukey test at the level of 5 %.

The dry matter of tubers was significantly affected by the interaction between accession and bio-mulch application time. The *Mangu* accession treatment with bio-mulch application time of 8 WBP and 12 WBP resulted in the highest tuber dry weight of 41.17% and 40.13%, respectively. This indicates that the low tuber weight is inversely proportional to the tuber dry matter value. The higher the tuber weight, the lower the tuber dry matter content. According to Tappiban et al. (2020), dry matter is more influenced by genetic characteristics than the environmental condition. According to Misganaw and Bayou (2020), the dry matter content of tubers is 41.2 - 50.2%, with an average of 45%. The tuber dry matter is formed from the translocation of photosynthate to the roots (Sulistiono et al., 2020).

Cassava productivity is affected by the bio-mulch application time. The B1, B2 and B3 treatments showed an insignificant different value with the control treatment (Figure 2). The average productivity was 32 - 44 t/ha. The *Mangu* cassava without bio-mulch treatment showed the highest productivity of 44.6 t/ha. At the same time, the B4 treatment showed the most negligible productivity at 22-33 t/ha with an average of 29.5 t/ha. This indicates that the different bio-mulch application time had no effect on the cassava productivity, but could suppress the weed growth in cassava plantations. According to Mansaray et al. (2022), legumes that

are planted after cassava will increase the cassava productivity. According to Alves (2001), in achieving the maximum productivity, a plant must maintain a balance between the source and the sink.

The dry matter of tubers in the B3 and B4 treatments on *Mangu* accession showed the highest value, which was presumed that the bio-mulch application time on eight and twelve weeks before cassava planting had sufficient soil moisture, so the water requirement for cassava roots could still be met. According to Enesi et al. (2022), planting cassava at the end of the year, around October to December, forms more tuber dry matter than those produced at the beginning of the year. Drought in areas with low rainfall conditions can decrease the tuber dry matter <30% lower than in areas with higher rainfall and lower evapotranspiration (El-Sharkawy, 2004). In this study, the initial conditions for cassava planting received a relatively low rainfall from early March to May, with less than 300mm/month. This condition produced a lower tuber dry matter. According to Anikwe and Ikenganyia, (2018), the cassava growth will be slow under drought conditions, as marked by the stem segment shortage and tuber development termination.

According to Aye (2012) cassava can withstand drought by slowing the growth, not by puddles and floods, that cause tuber rot.

Table 3. Effect of bio-mulch application time and accession type on tuber dry matter and plant biomass weight at six months of harvest

Bio-mulch	Cassava accession			
	<i>Ketan Malang</i>	<i>Genjah bayam</i>	<i>IR Jonggol</i>	<i>Mangu</i>
Plant biomass weight (kg)				
B0	2.95	2.86	3.42 a	3.00 ab
B1	2.45	3.00	3.03 ab	2.90 ab
B2	2.80	3.01	3.22 ab	2.93 ab
B3	2.51	3.10	2.92 ab	2.71 ab
B4	2.38	2.99	2.92 ab	2.13 b
Dry matter of tuber (%)				
B0	32.79	37.04	28.73	38.56
B1	37.63	27.42	37.38	37.79
B2	34.77	34.62	40.10	28.14
B3	32.25	33.78	37.73	41.17
B4	32.41	34.47	37.13	40.34

Note: Means followed by the same letters in the same row are insignificantly different according to Tukey test $\alpha = 5\%$. B0: without bio-mulch (control), B1: 0-week application before cassava planting, B2: 4-week application before cassava planting, B3: 8-week application before cassava planting, B4: 12-week application before cassava planting.

Table 4. Correlation matrix between yield and components.

Traits	PH	SD	PB	TN	TL	TD	FW	DM
SD	0.50*	1						
WB	0.57**	0.78**	1					
TN	0.54*	0.53*	0.54*	1				
TL	-0.56*	0.22ns	0.01ns	-0.22ns	1			
TD	0.10ns	-0.41ns	-0.46*	-0.20ns	-0.42ns	1		
FW	0.29ns	0.37ns	0.34ns	0.72**	-0.08ns	0.20ns	1	
DM	0.27ns	0.16ns	0.00ns	-0.08ns	0.13ns	-0.30ns	-0.37ns	1
TY	0.14ns	0.54*	0.456*	0.52*	0.24ns	-0.04ns	0.61**	-0.21ns

Note: PH = plant height, SD = stem diameter, PB = plant biomass, TN = tuber number, TD = tuber diameter, FW = fresh weight, DM = dry matter content, TY = total tuber yield, *significant ($\alpha = 5\%$), and ** highly significant ($\alpha = 1\%$)

Plant height showed a positive correlation to stem diameter ($r = 0.50^*$), number of tubers ($r = 0.54^*$), and highly significant to plant biomass weight ($r = 0.57^*$). This condition presents a higher plant height, a greater cassava stem diameter, and plant biomass weight. A higher plant weight biomass indicates an increased crown's weight, including the mature leaves, as the plant product for photosynthesis to produce and assimilate.

Cassava stem diameter showed a significant correlation with the number of tubers ($r = 0.53^*$) and tuber productivity ($r = 0.54^*$). Also, it showed a robust correlation with plant biomass weight ($r = 0.78^{**}$). Therefore, increasing the diameter of cassava stems can significantly increase the biomass weight compared to leaf organs. Bulb weight is strongly correlated with number of tubers ($r = 0.72^*$) and tuber productivity ($r = 0.61^*$). Therefore, the greater number of tubers, the heavier tuber weight, and the higher tuber productivity. Cassava productivity shows a significant correlation with stem diameter ($r=0.54^*$), plant biomass ($r = 0.45^*$) and number of tubers ($r=0.52^*$). According to Enesi et al. (2022b), the production of cassava tubers is influenced by soil fertility, tillage, and weeding.

Conclusions

Based on the experimental results and discussion, it can be concluded that:

1. No interactions were found between the cassava accession type and bio-mulch application time on the growth and yield of cassava plants
2. Cassava accession treatments showed a significant difference in growth parameter components, namely plant height, stem

diameter and yield components, including tuber length and diameter.

3. The bio-mulch planting time on twelve weeks before planting cassava tends to reduce the product yield of four cassava accessions.

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Budiarto R · Dwinanda SR · Pakpahan HA · Komala M · Yusti

A review of sapodilla beneficial use, production status, and propagation technique in Indonesia

Abstract. *Manilkara zapota* (L.), commonly known as sapodilla, is one of the tropical plants originating from Central and South America that is still less popular in Indonesia than banana, citrus, mango. To gain its popularity, it is crucial to review the beneficial uses, existing production status, and propagation techniques of sapodilla. In general, sapodilla is used for either as table fruit or derivative food. Additionally, it contains various bioactivities in its fruit, stem, and leaves, such as antioxidant, antimicrobe, and antitumor activity; thus, it becomes very potential for pharmaceutical purposes. The existing production data of sapodilla determine the West Java Province as the biggest production area in Indonesia, with more than 20% contribution to the national level (38.250 tons annually). In more detail, the top production area at the village level with a local sapodilla cultivar, Sukatali sapodilla, is found in Sukatali village in Situraja Subdistrict, Sumedang District, West Java Province. Sapodilla can be propagated by using both reproductive system and vegetative methods. Vegetative propagation of grafting is commonly used to produce shorter juvenile and uniform seedlings. However, it highly depends upon the grafting type, season, and scion diameter. Literature search on grafting recommended modified cleft grafting in June-July, using *Chrysophyllum lanceolatum* and *Manilkara hexandra* as rootstock, and scion with a diameter of 5.02 mm, as the best practice of sapodilla propagation.

Keywords: Antioxidant · Grafting · *Manilkara zapota* · Rootstock · Sukatali

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Budiarto R^{1*} · Dwinanda SR^{2,3} · Pakpahan HA^{2,3} · Komala M^{2,3} · Yusti^{2,3}

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang km. 21 Jatinangor, Sumedang 45363, Indonesia

² Study Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang km. 21 Jatinangor, Sumedang 45363, Indonesia

³ Department of Research and Development, Agricultural Student Organization (HIMAGRO), Faculty of Agriculture, Universitas Padjadjaran, Jalan Raya Bandung Sumedang km. 21 Jatinangor, Sumedang 45363, Indonesia

*Correspondence: rahmat.budiarto@unpad.ac.id

Introduction

Sapodilla (*Manilkara zapota*) is an important horticulture commodity worldwide, although sapodilla's center of origin is recorded in tropical Central and South America (Gilly, 1943; Fayek et al., 2012). *Manilkara zapota* (L.) have various synonyms, such as *Achras sapota*, *Achras zapota*, *Manilkara achras*, *Manilkara zapotilla* and *Mimusopus manilkara* (Mohidin et al., 1992; Quattrocchi, 1999). The tree is tolerant to shade and can be used for edible sweet fruit and latex for gum production (Toledo-Aceves et al., 2009).

Its fruit is commonly found in several countries, with different vernacular names, such as dilly in Bahamas, sapoti in Brazil, sapote in Cuba, sapatiha in Dutch West Indies, chiku in India, sawo in Indonesia, chikoo in Malaysia, chicozapote in Mexico, nispero in Puerto Rico, lamoot in Thailand, ciku in Singapore, sapodilla in UK and USA (Karle & Dhawale, 2019). The sapodilla fruit is composed of nutritious soft and sweet flesh, thus it will be attractive to be consumed as a fresh fruit. The fruit was popularly known as the source of vitamin C (Kusmiyati et al., 2014), amino acids in the form of aspartic and glutamic acid (Hall et al., 1980), and flavonoids such as quercitrin, myricitrin, catechin, epicatechin, gallocatechin (Ma et al., 2003).

In Indonesia, the popularity of sapodilla as a table fruit is still less than other tropical fruit such as citrus, banana, and mango (Efendi and Budiarto 2022). Additionally, there are limited researches to investigate the uses and popularity of sapodilla. A previous study by Kusmiyati et al. (2014) reported the applied culture practice and production of sapodilla locally in three subdistricts in Java. Kusmiyati et al. (2017) also stated that the quality of sapodilla fruit is in response to post-harvest handling.

One of the locally famous varieties of sapodilla is Sukatali sapodilla, which refers to the name of the village of Sukatali, Situraja Subdistrict, Sumedang District as the center of its development (Saepuloh et al., 2022). Sukatali sapodilla is a local superior agricultural commodity that can be used as a supporting component for ecotourism (Setiawati & Yunita, 2020; Djuwendah et al., 2017). The further development of Sukatali sapodilla is still widely open to fulfil the need of domestic and international markets. Moreover, this variety has

equipped with geographical indication certification, which potentially become its strength in entering the high-level market, such as the modern or international market (Setiawati & Yunita 2020).

The effort to develop the production of Sukatali sapodilla should be achieved by research activity, such as the enrichment of sapodilla information database that elaborates on any aspect regarding sapodilla in general or even Sukatali sapodilla in specific. Thus, the present study was aimed to review the beneficial use and production status of sapodilla in Indonesia.

Discussion

Beneficial Use. Sapodilla is mainly served as table fruit in fresh raw form (Yee & Shukkoor, 2020). This fruit can be found in both modern and traditional markets. The tree bears flowers all year round; thus, sapodilla fruit can always be regularly found in the market. Sapodilla fruit is generally slightly oval, with relatively rough skin, brown, soft flesh, and sweet taste. Sapodilla fruit is rich in nutrients that are beneficial to human health (Padmavathi, 2018; Mehnaz and Bilal, 2017), such as sugar, protein, dietary fiber, tannin, saponin, minerals (copper, potassium, iron) and vitamins (ascorbic acid, niacin, folate, and pantothenic acid) (Miranda, 2022; Bangar et al., 2022). Consuming sapodilla fruit is reported to improve the walking capacity of older people due to its rich vitamin C and vitamin A content, which positively affects the body's antioxidant status (Leelarungrayub et al., 2019). It also improves body immunity, and help to maintain digestive and cardiovascular health (Miranda, 2022).

The sapodilla fruit pulp is previously reported to have a high antioxidant potential content (Karle et al., 2021; Leong & Shui, 2002). The post-harvest handling of sapodilla fruit highly affects the antioxidant level, especially storage treatment (Shui et al., 2004). Aside from fruit pulp, sapodilla's fruit peel has a high antioxidant potential (Karle et al., 2021). An earlier report also showed that fruit peel contains more bioactive compounds than its pulp (Gomathy et al., 2013). Moreover, the unripe fruit of sapodilla fruit could be used to treat diarrhea, after being smashed and diluted with water, and then used to treat diarrhea

(Mohidin et al., 1992). The presence of bioactivity in sapodilla fruit is related to its physicochemical compounds, such as tannins (Matthew & Lakshminarayana, 1969; Pontes et al., 2002; Shui et al., 2004), triterpenes (Hart et al., 1973), and flavonoid (Ma et al., 2003).

In addition to the mentioned beneficial uses of fruit, the stem of sapodilla can also be use as gummy latex for chewing gum production (Ma et al., 2003) and pharmaceutical material. The stem bark of sapodilla possesses several bioactivities, such as antitumor activity (Osman et al., 2011a), antibiotic, astringent (Kaner et al., 2009), and antimicrobial against several pathogenic bacteria (*Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Sarcina lutea*, *Escherichia coli*, *Shigella sonnei*, *S. shiga*, *S. dysenteriae*, and *Salmonella typhi*) and fungi (*Aspergillus flavus*, *Vasianfactum sp.*, and *Fusarium sp.*) (Osman et al., 2011b).

Not only fruit and stem but also the sapodilla leave is reported to have beneficial bioactivities, such as an antioxidant (Chanda & Nagani, 2010), antimicrobe (Nair & Chandra 2008; Kaner et al., 2009), antitumor (Rashid et al., 2014), analgesic (Jain et al., 2011), antihyperglycemic and hypocholesterolemic (Fayek et al., 2012). The leaves are previously reported to treat cold, cough, and diarrhea (Ma et al., 2003). Kaner et al. (2009) reported the presence of less alkaloid, less flavonoid, high tannin, moderate phlorotannins, high triterpenes, no steroid, moderate saponin, and less cardiac glycosides in the leaf of sapodilla.

Production status. Sapodilla's production status in Indonesia is illustrated in Figure 1 (BPS, 2020). Numerous provinces throughout Indonesia produce this fruit. The top five lowest sapodilla production in 2020 was found in North Sulawesi (2 tons/year), North Maluku (7 tons/year), Papua (20 tons/year), Maluku (31 tons/year), and Aceh (46 tons/year). On the opposite, the top five highest sapodilla production was achieved by West Java (38.250 tons/year), North Sumatra (19.886 tons/year), East Java (19.898 tons/year), Lampung (19.371 tons/year), and Central Java Province (19.294 tons/year). In 2020, there was a total of 186.706 tons/year of sapodilla produced by Indonesian, and 21% of them is the contribution of West Java Province (BPS, 2020). One of the production districts in West Java province was Sumedang. The top four sapodilla production subdistricts in 2012 were Situraja (6886 tons), Darmaraja (5168

tons), Cisitua (4881 tons), and Ganeas (3861 tons) (BPS Kabupaten Sumedang 2012). Situraja, the highest sapodilla production subdistrict, is popular with its local sapodilla variety, Sukatali sapodilla.

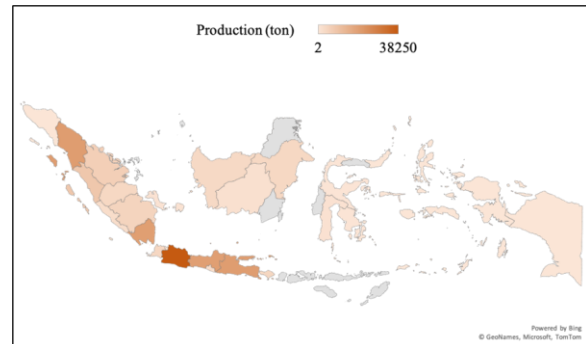


Figure 1. The sapodilla production (tons) map throughout Indonesia (BPS, 2020).

Note: Brown color gradation implied the sapodilla fruit production (tons) data throughout Indonesia in 2020

Culture practices. Sapodilla can be cultured in monoculture and polyculture system. Earlier study used to mix sapodilla cropping with ginger and turmeric (Pandey et al., 2016). In contrast, previous research by Kusmiyati, et al. (2014) has reported the local monoculture practice of sapodilla cultivation in Trirenggo Village, Bantul Regency, Yogyakarta Province. Traditionally, traditional cultivation practices begin with seeding, land preparation, planting, maintenance, and harvesting. Air layering propagation techniques prepare seedlings of sapodilla. The mother plant used for air layering is a local sapodilla tree grown around the village. Land preparation and planting are conducted during the rainy season by making planting holes measuring 50 cm in length x 50 cm in width x 50 cm in depth (Kusmiyati, et al. 2014). Sapodilla seedling is transplanted amid the local garden that accommodates a polyculture system. Local farmers need to use regular plant spacing. Farmers carry out watering, organic fertilization, and less intensive pest-disease control at the maintenance stage (Kusmiyati, et al. 2014). To control weed invasion and growth, farmers of sapota can apply soil cover (Reddy & Khan, 2000). Integrated nutrient management to improve sapota growth is recommended in the form of NPK fertilizer (1000:1000:1500 g NPK tree-1), vermicompost, and activated effective microorganism (Tasleema et al., 2019). Plant growth regulator in form of paclobutrazol could

stimulate the increase of fruit yield of sapodilla (Reddy & Khan, 2001). Harvesting is usually done in a period of 1-2 weeks. Marketing rests on the existence of the middleman. The annual productivity of sapodilla plants is around 50-70 kg of fruit per tree, with the selling price at the farmer gate around IDR 100,000 per tree.

The uniqueness of sapodilla fruit is found in its extremely susceptible character to perishability on ripening (Singh et al., 2021). Thus, post-harvest handling is crucial to indicate the quality of fruit by farmers or collectors. Post-harvest is one of several critical points in sapodilla fruit production. The less accurate post-harvest handling caused a significant loss of sapodilla fruit in terms of quality and quantity. One of several essential issues in sapodilla is how to prolong the shelf life of sapodilla fruit (Raesi, 2013).

Sapodilla fruit can only be stored for 6 days at room temperature (27-29°C, Rh 65-75%); however, chitosan coating treatment with a concentration of 1.5 – 3.0% can extend the shelf life 2 days longer (Kurniawan et al., 2013). Combining chitosan and 20% beeswax is recommended to extend sapodilla fruit shelf-life (Foo et al., 2018). In addition, Hasmorro et al. (2014) reported that fruit immersion in a solution of 4% and 6% of CaCl₂ could prolong up to 10 days.

Product diversification is also applicable to sapodilla to improve the likeliness of sapodilla and give added value to sapodilla fruit commodities. Sapodilla can be processed as jam, syrup, and vinegar to increase its shelf life and value-added (Raesi, 2013).

Propagation techniques. Sapodilla propagation can be carried out in both sexual and vegetative methods. Fertilization is required between male and female flower organs, to produce sapodilla fruit containing seeds for planting material. In contrast to reproductive methods that produce less uniform and labile genotypes with a longer juvenile period, the vegetative method has more uniform and stable planting materials with a shorter juvenility (Kaur et al., 2020). Various techniques to vegetatively propagate sapodilla can be achieved in the form of grafting and air layering, while cutting could be more effectively applied even with the plant growth regulators supplementation (Chadha, 1992). Air layering is a more effortless and quicker method; however, it produces shallow-rooted plants prone to

falling when exposed to high wind. In contrast, grafting is a promising method that has a deeper root structure due to the presence of rootstock.

The rootstock is crucial for grafting because it determines the scion canopy's growth and harvested yield (Mukherjee & Litz, 2009). Several rootstocks are commonly used to support the production of sapodilla-grafted seedlings, namely *Chrysophyllum lanceolatum* (Kalesh et al., 2005) and *Manilkara hexandra* (Kaur et al., 2020). In a particular case, the seed of *Manilkara hexandra* requires seed priming treatment to improve the seed germination response (Bhanuprakash et al., 2008; Reddy & Khan, 2002). One of the popular seed-priming chemicals to increase seed vigor is gibberellic acid (Kaur et al., 2020; Bajaniya et al., 2018; Ballantyne, 1991) which regulates starch mobilization used for respiratory substrate (Shah, 2007) and then boosts the biosynthesis of proteolytic enzymes like alpha-amylase (Prasad & Prasad, 2009) and ribonuclease to hydrolyze starch in the endosperm providing the sugars for the initial germination processes (Copeland & Mc Donald, 1995).

Aside from plant growth regulator like gibberellic acid, numerous previous studies revealed the crucial factor of grafting type and season in determining the success of grafting in sapodilla. Islam et al. (2004) decided that modified cleft grafting as the best type of grafting rather than cleft and veneer grafting, with a mean of graft survival of about 94%, 89%, and 78%, respectively. While Shirol et al. (2005) recommended the inarching method due to its maximum graft success, especially in June-July, due to the minimum fluctuation of relative humidity and temperature during that period. Similarly, softwood grafting performs the best in July with the best graft survival rate (Wazarkar et al., 2009; Ghosh et al., 2010), whereas the minimum graft survival rate is recorded in September-October (Ghosh et al., 2010). Maske, et al. (2010) also confirm poor graft survival rate on plants grafted from November to April. The culture of softwood sapodilla grafting seedlings under moderate shading results in the number of days to bud sprout data, ranging from 14 to 18 days (Kalalbandi et al., 2014). Nitish et al. (2019) reported a faster sprout coming when the grafting is performed in July rather than August. Numerous studies also said the best growth on seedlings as the effect of grafting in July (Nitish et al., 2019; Ghritlahare & Ashutosh, 2018;

Niranjan, 2011; Wazarkar et al., 2009). Aside from seasonal variation, earlier studies also reported the effect of the scion diameter on the success of sapodilla grafting. The scion with a diameter of 5.02 mm was recorded to have a greater graft success rate than 4.63 mm, 4.38 mm, and 4.02 mm scion diameter (Tanjua & Thippesha, 2016). These results shows that more food reserves in the scion, with a large scion diameter indicator, increase the success of post-grafting recovery growth and *vice versa*.

Conclusion

Sapodilla has numerous beneficial uses, such as table fruit and pharmaceutical material, due to the various bioactivities in its fruit, stem, and leaves, such as antioxidant, antimicrobe, and antitumor activity. The production of sapodilla is found in numerous provinces in Indonesia, with West Java as the top production area. Sukatali Village in Situraja Subdistrict, Sumedang District, West Java, is determined as the top production area at the village level with a local sapodilla cultivar, namely Sukatali sapodilla. Sapodilla can be propagated by using both sexual reproduction and vegetative methods. Vegetative propagation of grafting is commonly used to produce shorter juvenile and uniform seedlings, and it is highly dependent upon the grafting type, grafting season, and scion diameter.

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Nugraheni · Pujiasmanto B · Samanhudi · Amalia TS

Comparison between the electrical conductivity method and radicle emergence test as a rapid test of sorghum seed vigor

Abstract. Sorghum belongs to multipurpose crops. All parts of sorghum can be used both as main products and derivative products; some derivative products produced from the sorghum plant consist of sugar, bioethanol, biomass, handicraft raw materials and starch. This study aims to determine the time required for testing the vigor and viability of sorghum seeds using the electrical conductivity and radicle emergence methods. The study was conducted in two experimental stages using a completely randomized design. The first experiment consisted of two factors: the number of sorghum seeds (40, 70 and 100 grains) and the volume of soaking water (75, 100, 125, and 150 ml). The second experiment consisted of two factors: research method (germination/control method, electrical conduction method, and radicle emergence method) and varieties (consisted of Numbu, Kawali, Suri 3, and Suri 4). The first experiment's results showed that the best electrical conductivity method on Suri 4 varieties were 40 seeds and 150 ml water volume. The electrical conductivity value is negatively correlated with Germination capacity, vigor index, growth of speed, maximum growth potential, and sprout growth rate. Time needed for Electrical Conductivity method in this study was three days. The germination period of 96 hours gives the best results on the radicle emergence of sorghum seed varieties. Vigor index, growth of speed, germination capacity, and maximum growth potential are positively correlated with the value of radicle emergence.

Keywords : Electrical conductivity · Germination · Radicle emergence · Viability · Vigor

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Nugraheni^{1*} · Pujiasmanto B¹ · Samanhudi^{1,2} · Amalia TS¹

¹ Master Program of Agronomy, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia

² Center for Research and Development of Biotechnology and Biodiversity, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia

*Correspondence: heni0336@gmail.com

Introduction

Sorghum contains various vitamins, including 0.09 mg of vitamin B1, 0.14 mg of vitamin B2, vitamin B3 as much as 2.8 mg and minerals such as iron 4.4 mg, sodium 7 mg, potassium 249 mg, calcium 28 mg, phosphorus 287 mg and contains relatively high carbohydrates 73.10-79.12% and Protein of 7.98-11.77% (Widowati and Luna, 2022).

Sorghum belongs to multipurpose crops. All parts of sorghum can be used both as main products and derivative products. The main products of the sorghum plant are seeds, leaves and stems, while some derivative products produced from the sorghum plant consist of sugar, bioethanol, biomass, handicraft raw materials and starch. (Subagio and Suryawati, 2013).

Seeds are one of the most important production factors in agricultural cultivation because they can affect the level of production achieved so that quality seeds are needed in the cultivation of sorghum plants. Quality seeds guarantee physical, genetic, and physiological qualities (Ilyas, 2012). The estimation of the physiological quality of a seed is the high and low viability of seeds which is reflected in the germination value, growing speed, and cohesiveness of growth (Widajati et al., 2013).

One of the influencing limiting factors development and production sorghum in Indonesia is the provision of quality sorghum seed. The providing of quality sorghum seeds is influenced by seed storage. Duration of storage and room temperature are the main factors that cause seed deterioration and seed vigor (Pramono et al., 2019).

Sorghum seeds have a fairly high carbohydrate and protein content, which causes the rapid decline of sorghum quality (Susilowati and Saliem, 2013). The deterioration of sorghum seeds during storage signals that the vigor of the seeds is low.

Deterioration is a life process leading to the deterioration of seeds even irreversible death. Seed deterioration can be viewed biochemically and physiologically. Biochemical indications in deteriorated seeds can be characterized by the occurrence of a decrease in enzyme activity, decrease in feeding reserves and increase electrical conductivity value. While physiological indications can be seen from the presence of changes in seed color, delayed seed germination,

decreased growth rate of sprouts, reduced germination, as well as increased abnormal sprouting (Hartati, 2019).

Germination testing for sorghum seeds is 10 days. This test is considered inefficient for quick and continuous provision of sorghum seeds, so it is necessary to increase the efficiency of the seed vigor test in order to shorten the testing time through a fast, cheap and applicable method. Innovation in the development of seed quality testing methods needs to continue to be carried out to obtain test results in a short time/efficiently (Suhartanto, 2021).

Vigor testing of seeds that have been validated by International Seed Testing Association (ISTA) to obtain results in a relatively short time is radicle emergence method and electrical conductivity method. The principle of the electrical conductivity method is a physical seed test that reflects the leakage rate of the cell membrane (ISTA, 2021). The principle of the radicle emergence method is when the radicles have appeared at least 2 mm long (Astuti et al., 2020). The Radicle emergence method is characterized by the rate of radicle emergence at the beginning of germination, which is an indication of the vigor of a seed (Yukti et al., 2018). Radicle emergence (RE) is a rapid vigor test recommended by International Seed Testing Association (ISTA) in 2021 for corn seeds at $20 \pm 1^{\circ}\text{C}$ for 66 hours ± 15 minutes or at a temperature of $13 \pm 1^{\circ}\text{C}$ after 144 hours ± 1 hours, radish seed (*Raphanus sativus*) at $20 \pm 1^{\circ}\text{C}$ for 48 hours ± 15 minutes, *Brassica napus* (oil seed rape, *Argentine canola*) at $20 \pm 1^{\circ}\text{C}$ temperature for 30 hours ± 15 minutes and *Triticum aestivum* L. *Subsp aestivum* at $15 \pm 1^{\circ}\text{C}$ temperature for 48 hours ± 15 minutes (ISTA, 2021).

Vigor testing is accepted as an official method in ISTA regulation of 2011 is conductivity testing for *Pisum sativum*, *Phaseolus vulgaris* and *Glycine max* (ISTA, 2011). Conductivity test can predict the field emergence and standard germination. Electrical conductivity test has been proved as indicator of seed vigor in wide range of crop species and is related to field emergence and stand establishment (Fatonah et al., 2017). Radicle emergence test is considered as a quick test to predict varying vigor level and field performance of seed lots than the standard germination test in several crops (Matthews and Powell, 2011).

The purpose of the study was to obtain the fastest level of time efficiency in Radicle emergence method compared to germination

method and electrical conductivity method in vigor testing and viability of sorghum seeds.

Materials and Methods

Research Materials and Tools. This research was carried out in June – October 2022 at the Laboratory of the Center for Supervision and Certification of Horticultural Food Plant Seeds in Central Java Province. In addition, potassium, magnesium and sodium leakage was conducted at Testing Laboratory of the Department of Agricultural Industrial Technology, Bogor Agriculture University. The materials used include sorghum seeds four varieties, aquadest, frosted paper, aluminum foil, label paper, tissue, plastic, cow manure, soil, polybags, ultraviolet plastic thickness 200 microns, and bamboo.

The tools used in the study consisted of glass jar cups, Eutech Instrument Conductivity meter Con 110, measuring cups, plastic tubs, germinators temperature $25 \pm 2^{\circ}\text{C}$, divider, analytical scales, grinders, ovens, desiccators, aluminium dishes, tweezers and rulers.

Methods. The study was conducted in two experimental stages consist of:

- a. Experiment I aim to determine the right combination of treatment for the number of seeds and the volume of soaking water in the electrical conductivity test of sorghum seeds which is arranged in completely randomized design consisting of 2 factors repeated 4 times. The first factor is the number of sorghum seeds consisting of 3 levels (40, 70 and 100 seeds), the second factor is the volume of soaking water consisting of 4 levels (75, 100, 125 and 150 ml of aquadest) so that 12 treatments combinations are obtained.
- b. Experiment II, i.e., seed vigor test, was carried out using the completely randomized design which was factorially arranged with 2 factors and repeated 4 times. First factor was test method consisting of three levels (seed germination method (Control/ M_0), electrical conductivity method (M_1) and radicle emergence method (M_2), the second factor is sorghum seed varieties consisting of 4 levels (V_1 : Numbu, V_2 : Kawali, V_3 : Suri 3 and V_4 : Suri 4) repeated 4 times, resulting in 48 treatment combinations. Vigor and viability testing of sorghum seeds using test media between paper rolls as many as 50 sorghum seeds were repeated 8 times with 3 sheets of

planting media, germinated at a standard temperature germinator of $25 \pm 2^{\circ}\text{C}$ (Pujiasmanto, 2017).

Observational modifiers, including:

- a) Vigor index (VI), percentage between the number of normal seedling at first count (four days after seedling) and the total number of seeds planted
- b) Growth speed (GS), the calculation of the percentage number of normal sprouts on the first day until the last observation of testing
- c) Electrical conductivity test (EC).

EC value is calculated using the following formula:

$$EC = \frac{(EC \text{ seed} - EC \text{ blank value})}{(\text{Weight (gram)})}$$

- d) Leakage analysis, this analysis is performed by soaking 50 sorghum seed in 250 ml of aquadest for 24 hours; further, leakage of ion potassium, magnesium and sodium ions present in water are measured using tool named *DHL Horiba*.
- e) Radicle emergence test, the calculation of the radicle emergence test is carried out every 24 hours, then the percentage of the number of seeds that have appeared radicles with the criterion of the minimum length of the radicle that grows is 2 mm.
- f) Mean germination time (MGT)
MGT testing is carried out to determine the average germination time of seeds starting from imbibition to the emergence of radicles of at least 2 mm (Matthews and Khajeh, 2006). MGT testing is performed to determine the average germination time of seeds. The average germination time is calculated by the formula:

$$MGT = \frac{(\sum nx t)}{\text{total germinated seeds}}$$

Note :

n = the number of germinated seeds at time t
t = hour after germination
(Astuti et al., 2020)

- g) Germination capacity, the ability of seeds to germinate normally at the first count (4 days after planting) and the final observation of testing (10 days after planting) divided by the total number of seeds planted
- h) Maximum growth potential, the overall percentage of sprouts that grew both normal and abnormal sprouts until the end of the observation.

- i) Sprout growth rate, the dry weight of seedling divided by the number of normal seedling in the final calculation of germination observation.

Data Analysis. The data obtained were analyzed using analysis of variance ($\alpha = 5\%$) and continued with Duncan Multiple Range Test ($\alpha = 5\%$). Coefficient correlation is performed to calculate the closeness of the relationship between EC and RE values with various observed vigor test benchmarks.

Results and Discussion

Based on Figure 1 of the electric conductivity test results showed that the treatment 40 seed, 75 ml aquadest in Kawali variety has the highest electrical conductivity value of 58.1 $\mu\text{S}/\text{cm.g}$, while the lowest electrical conductivity value was obtained from the treatment 40 seed, 150 ml aquadest in Suri 4 variety was 11.4 $\mu\text{S}/\text{cm.g}$.

The electrical conductivity is a vigor test whose principally based on integrity of cell membranes. Electrical conductivity done by measuring electrolyte leaking from seed tissue is dissolved into seed soaking water due to leakage of cell membranes (ISTA, 2014). Low seed vigor will indicate a high electrical conductivity value, otherwise high seed vigor will indicate the membrane leakage value or electrical conductivity value was low (Widajati et al., 2013). In Figure 2 showed that the Kawali variety

has the highest Potassium (K) value 3.31 mg/L, and Sodium (Na) ion leakage value 0.303 compared to other varieties, and the Suri 4 variety in a addition to having the lowest electrical conductivity value in figure 1, also has the lowest ion membrane leakage ions compared to other sorghum varieties. According Andini et al. (2021) damage to cell membrane in seed result in leakage of sugars and electrolytes which has an impact on decreasing metabolic and transportation efficiency. The higher the electrical conductivity value, the higher the membrane leakage. Changes in membrane integrity are early symptoms of the seed deterioration process resulting in the release of compounds from the seed which are observed based on electrical conductivity and concentration of metabolite compounds (sugars, amino acids, fatty acids, enzymes, inorganic ions such as K^+ , Na^+ , Ca^{2+} and Mg^{2+} (Vieira et al., 2008).

Based on Table 1 it showed that Suri 4 variety sorghum seeds have significantly different effects on vigor parameters and seed viability but have average electrical conductivity value did not significantly different in other variety. Khairani et al. (2022) reported that a high indicates that seed vigor is low; this is the opposite way around that a low electrical conductivity value has a high vigor and viability value. This is indicated by the high germination value. Seeds germinate faster, classified in seeds with strong vigor. Growing speed testing is one of the seed vigor tests. Seeds with high vigor are able to grow faster than seeds that are less vigorous (Widajati et al., 2013).

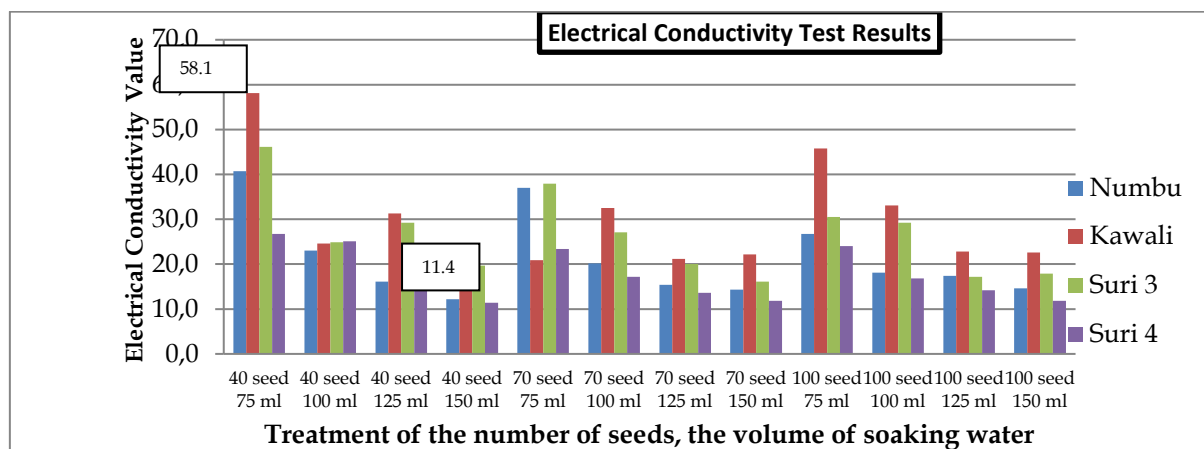


Figure 1. Electrical Conductivity Test Results Consisting of Twelve Treatment Combinations of Number of Seeds and Volume of Soaking Water

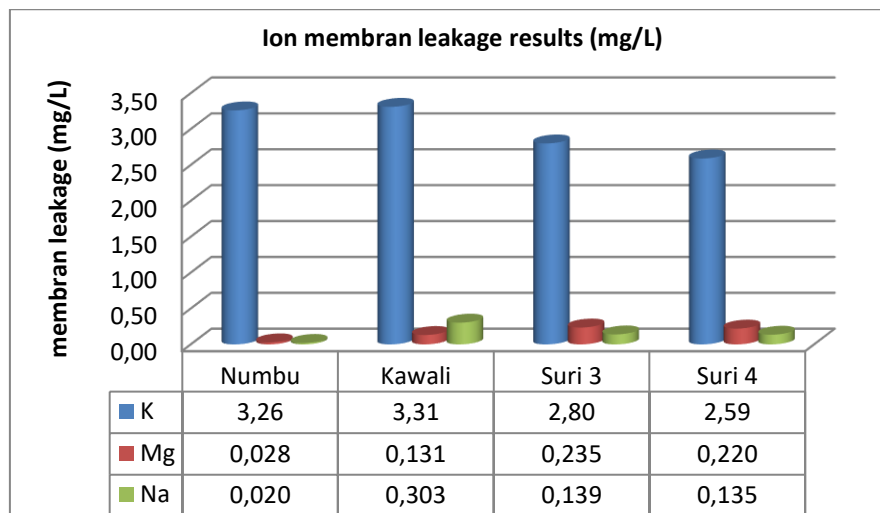


Figure 2. Ion Membrane Leakage Value of Electric Conductivity Test Bath Results on 4 Varieties of Sorghum Seeds

Source: Analysis of the Testing Laboratory of the Department of Agricultural Industrial Technology IPB

Table 1. Value of Electrical Conductivity, Germination Capacity, Vigor Index, Growth of Speed, Maximum Growth Potential and Sprout Growth Rate of 4 sorghum varieties.

Varieties	Average					
	Germination Power (%)	Vigor Index (%)	Growth of Speed (%) Normal Seedling/etmal)	Electrical Conductivity (μS/cm.g)	Maximum Growth Potential (%)	Sprout Growth Rate (mg/Normal Seedling)
Numbu	84.50b	25.75a	19.02a	21.30 a	89.25b	11.58b
Kawali	82.75b	20.50a	17.26a	29.40 a	90.75b	9.8a
Suri 3	76.00a	30.0ab	17.43a	26.37 a	82.75a	9.24a
Suri 4	91.75c	39.25b	21.63b	17.65 a	97.25c	11.72b

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 2. The Results of the Correlation Analysis between Electrical Conductivity EC) with Germination Power (GP), Vigor Index (VI), Growth of Speed (GS), Maximum Growth Potential (MGP) and Sprout Growth Rate (SGR) and Growth Test (GT).

	GP	VI	GS	MGP	SGR	GT	EC
GP	1						
VI	0.353	1					
GS	0.678**	0.755**	1				
MGP	0.880**	0.262	0.557*	1			
SGR	0.621*	0.495	0.635**	0.444	1		
GT	ns	ns	Ns	ns	ns	1	
EC	-0.179	-0.360	-0.530*	-0.108	-0.397	ns	1

Description: r = correlation coefficient, * = real effect on 5% test, ** = very real effect on 1% test, ns = not real significant 5% level

The correlation coefficient (r) of each test in Table 2 is obtained from the results of the correlation test between the value of electrical conductivity to vigor modifiers and seed viability and gives negative or negatively correlated results. The value of Electrical Conductivity

correlates very low with the germination power variable with the value of the correlation coefficient (r) -0.179, low correlated with the vigor index variable with the value of the correlation coefficient (r) -0.360, correlated quite strongly with the variable growth speed with the value of

the correlation coefficient (r) -0.530, very low correlated with the maximum growth potential variable with the value of the correlation coefficient (r) -0.108 and low correlated with the variable sprout growth rate with the value of The correlation coefficient (r) is -0.397. The negative correlation value is shown between the value of electrical conductivity with vigor modifiers and seed viability. In accordance with the opinion Chhetri (2009) that seeds with low germination and vigor provide high electrolyte leakage, in contrast to high-vigor seeds with low electrolyte leakage. The correlation relationship between the electrical conductivity test and the germination value, growth power, vigor index and tetrazolium of wheat seeds in the study Murwantini (2013) provides negative correlation results which show that the higher the value of electrical conductivity, the lower the value of germination power, growing power, vigor index and tetrazolium.

Based on Table 3 and Table 4 shows that the treatment of the Radicle emergence method has a significantly different influence on the germination method (control) and the electric conductivity method. The radicle emergence (M_2) method gives the highest vigor index result of 45.06%, germination method 34.75% and electric conductivity method 28.88%. High radicle emergence calculations at the beginning of germination indicate high seed vigor, while low radicle emergence indicates low seed vigor. The radicle emergence test time is faster than other vigor methods because the calculation of radicle emergence is done earlier, which is when the radicles have appeared at least 2 mm long. Calculation of high radicle emergence indicated

high seed Vigor (ISTA, 2014). The results of Table 4 showed that Vigor Index of Suri 4 varieties have a significantly different influence on Suri 3, Kawali and Numbu varieties. The vigor index value in the Suri 4 variety is 47.17%. According to Dwipa and Saswita (2017) the initial growth of seeds is more influenced by the genetic ability of seeds, so vigor and viability are strongly influenced by genetic factors. The Suri 4 variety is able to germinate faster compared to other varieties. A high vigor index value indicates that the seeds germinate faster, so the Suri 4 variety is classified under strong vigor.

Table 3. Mean of Vigor Index Values on different test method treatments.

Test Method	Vigor Index
Germination Method (M_0)	34.75 a
Electrical Conductivity Method (M_1)	28.88 a
Radicle Emergence (M_2)	45.06 c

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 4. Mean of Vigor Index Value in different varieties.

Varieties	Vigor Index
Numbu (V_1)	29.67 a
Kawali (V_2)	31.25 ab
Suri 3 (V_3)	36.83 b
Suri 4 (V_4)	47.17 c

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 5. Mean of Radicle Emergence (RE) at each observation period.

Varieties	Mean of radicle emergence (%)				
	24 hours	48 hours	72 hours	96 hours	120 hours
Numbu	66.75 a	82.25 a	82.75 a	86.00 a	88.00 a
Kawali	78.25 b	89.50 b	92.75 b	95.00 b	95.00 b
Suri 3	65.00 a	80.00 a	80.50 a	83.50 a	83.50 a
Suri 4	81.25 b	92.25 b	92.50 b	95.25 b	95.25 b

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

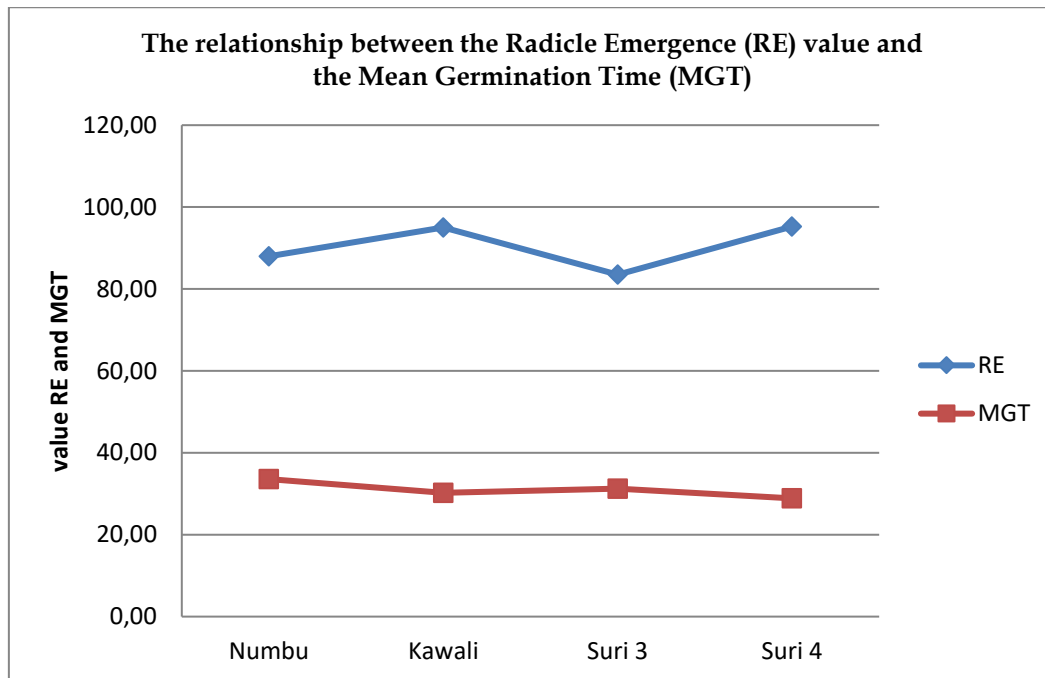


Figure 4. The Relationship between the Radicle Emergence and Mean of Germination Time

The mean germination time is the time needed to germinate from imbibition to the emergence of radicles at least 2 mm long. Roots with a length of ≥ 2 mm can already be called germinated (Luo et al., 2015). Observations of radicle emergence (RE) were carried out at a temperature of $25 \pm 2^{\circ}\text{C}$ with germination times ranging from 24 hours to 120 hours. The determination of the observation time was carried out to see the difference in radicle emergence values for each observation period in each sorghum variety. Based on Table 3 shows that the most radicles appear in the first 24 hours followed by the next 48 hours. Determination of the observation time of radicle emergence can be done by correlating the radicle emergence in each observation period with the mean of germination time. According to research Noeryanti et al., (2022) the mean of germination time describes the average time for seeds to germinate from the beginning of imbibition to the emergence of radicles. The lower the average value of germination time, the faster the seeds germinate.

Table 6 shows that using the Radicle Emergence method the Suri 4 (M_2V_4) variety provides the highest percentage of growth in the variables of growing speed, germination capacity and maximum growth potential with a growth speed value of 26.32% Normal Seedling/ etmal, germination value: 97.50%, maximum growth

potential: 98.50%. According to Kartasapoetra (2003) there is a close relationship between the speed of growing and the vigor of seeds. Seeds that have a high growth rate will be more resistant to conditions or environments that are not suitable (suboptimum). Measurement of growing speed is carried out by summing the normal growth of sprouts daily or ethmal during the germination period. The value of growing speed indicates the vigor of seeds under sub-optimal environmental conditions, assuming fast-growing seeds are able to overcome sub-optimal conditions (Sadjad, 1993). The difference in germination value of each variety is strongly influenced by genetic factors of each sorghum variety, this as stated by Justice and Bass (1990) interspecies variation affects the shelf life of seeds. Calculation of the appearance of high radicles indicates high seed vigor. Some of the advantages of the Radicle Emergence method are that the testing method is fast, the procedure is simple and can predict seed vigor and obtain preliminary information in predicting normal germination of seeds compared to the germination method (Matthews and Powell, 2011). Seed lots that need a longer time between imbibition and Radicle emergence, as seen in low vigor lots, are necessary to allow for metabolic repair. In high quality seeds, Radicle emergence earlier since there is much less ageing induced

damage need DNA repair (Demir et al., 2022). The Suri 4 variety is classified as a seed that has high vigor and viability. This is in accordance with the opinion Ilyas (2012) seeds that have high viability are characterized by maximum growing potential, high germination and dry weight, while seeds with high vigor are characterized by high growing speed and high growing ability in a suboptimal environment. The maximum growth potential is one of the determinants of seed viability which is greatly influenced by seed quality and maximum seed growth limits. Seed quality is largely determined by environmental and genetic factors (Fitriani et al., 2021). Maximum Growth Potential is the percentage of all seeds that live or show symptoms of life both producing normal and abnormal sprouts, namely

the potential for a seed lot where seeds can have maximum germination (Hartati, 2019). The sprout growth rate is the dry weight of the sprout divided by the number of normal sprouts until the end of the observation. Control treatment (germination method), Suri 3 variety gave the highest sprout growth rate yield of 15.48% compared to other treatments. Normal sprout dry weight is an indicator of seed vigor, a high normal sprout dry weight value indicates a high vigor value.

The highest normal sprout value, which is an indicator that affects the total dry weight of sprouts. This is in consistent with the opinion Dehnavi et al. (2020) that differences in germination parameters in sorghum seeds are influenced by genetic variation between sorghum genotypes.

Table 6. Mean of interaction of test method and sorghum seed varieties on Growing Speed, Germination, Maximum Growth Potential, and Sprout Growth Rate of 4 sorghum varieties.

Treatment	Mean			
	Growth of Speed (%Normal Seedling/etmal)	Germination Capacity (%)	Maximum Growth Potential (%)	Sprout Growth Rate (mg/ Normal Seedling)
M ₀ V ₁	15.38 a	80.00 abc	90.25 cde	12.63 de
M ₀ V ₂	19.60 cd	84.75 bcd	87.25 bc	10.60 bc
M ₀ V ₃	16.44 ab	74.75 a	77.00 a	15.48 g
M ₀ V ₄	22.77 e	95.00 fg	96.25 efg	14.32 fg
M ₁ V ₁	18.18 bc	84.50 bcd	89.25 cd	11.58 cd
M ₁ V ₂	17.26 abc	82.75 bcd	90.75 cde	9.8 ab
M ₁ V ₃	17.43 abc	76.00 a	82.75 b	9.24 a
M ₁ V ₄	21.63 de	91.75 efg	97.25 fg	11.72 cd
M ₂ V ₁	18.71 bc	78.75 ab	91.75 cdef	13.68 ef
M ₂ V ₂	19.78 cd	88.50 def	95.25 defg	10.84 bc
M ₂ V ₃	21.27 de	86.75 cde	91.25 cdef	13.43 ef
M ₂ V ₄	26.32 f	97.50 g	98.50 g	14.59 fg

Note: M₀: Control (Germination Method), EC : Electrical Conductivity Method and RE : Radicle Emergence Method. V₁ : Numbu, V₂ : Kawali, V₃ : Suri 3 dan V₄ : Suri 4. Numbers followed by the same letters in the same column are not significantly different based on the 5% DMRT test

Table 7. Results of correlation analysis between Radicle Emergence (RE) value with variables VI, GS, G, MGP, SGR and MGT.

	RE	VI	GS	GC	MGP	SGR	MGT
RE	1						
VI	0,505*	1					
GS	0,439	0,878**	1				
GP	0,336	0,548*	0,831**	1			
MGP	0,361	0,336	0,599*	0,824**	1		
SGR	-0,062	0,646**	0,625**	0,230	0,70	1	
MGT	-0,202	-0,188	-0,476	-0,726**	-0,519*	0,068	1

Description: r = correlation coefficient, * = real effect on 5% test, ** = very real effect on 1% test, tn = no real at 5% level, VI = vigor index, GS = growth speed, MGT (mean germination time), GC = germination capacity, MGP = maximum growth potential and SGR = sprout growth rate

Based on Table 7 shows that the variables of vigor index, growth of speed, germination power and maximum growth potential have a positive correlation with the value of radicle emergence, while the variables of mean germination time and sprout growth rate show a negative correlation with the value of radicle emergence. According Kusumawardana (2019) the highest of radicle emergence had positive correlation with germination, vigor index, speed of growth, and field emergence. Research by Noeryanti et al (2022) reported that radicle emergence test had a high positive correlation with seed viability test for germination, seed vigor index, growth of speed, dry weight of germination normal and germination growth rate, and also give high negative correlation with mean germination time.

Conclusion

- a. The time needed to determine the value of electrical conductivity is three days. The value of electrical conductivity is negatively correlated with vigor and viability parameters. The higher the germination power, vigor index, growth of speed, maximum growth potential and sprout growth rate, the lower the Electrical conductivity value
- b. The observation time for radicle emergence (RE) testing is four days. The most radicles emergence in the first 24 hours, followed by the next 48 hours until the end of observation, which is 96 hours (4 days). The Suri 4 variety has the smallest average germination time of 28.87 hours, having the highest radicle emergence rate of 95.25%.
- c. The variables of vigor index, growth of speed, germination power and maximum growth potential are positively correlated with the value of radicle emergence, while mean germination time and sprout growth rate are negatively correlated with the radicle emergence value.

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Zaw ZN

A review of low-frequency latex harvesting systems that lessen the tapper shortage problem of the smallholders' natural rubber production

Abstract. Smallholders' rubber production is encountering problems of skilled tapper shortage and high production costs resulting from increased worker wages and the substantial growth of new mature areas. Low-frequency latex harvesting system (LFLHS) effectively improves tapper productivity with long-term optimum yield by reducing the tapper requirement. LFLHS reduces tapper requirement by 33% to 67% of the conventional harvesting systems. Under the d3 (tapping every three days) frequency harvesting system, a tapper is assigned to cover three tasks, and his productivity is at least 30% higher than that of the d2 (alternative daily) frequency harvesting system. The cumulative yield of LFLHS is comparable to that of d2 frequency. It is economically profitable when the cumulative yield of LFLHS reaches 90% of the d2 frequency tapping as a break-even yield. Its low number of tapper requirement and high productivity saves tapping cost. 20% to 55% of tapping cost can be reduced by shifting the harvesting frequency from d2 to d3. The virgin bark of basal panels could be tapped at least four to ten years more than conventional tapping systems. The low bark consumption allows sufficient time for the regeneration of bark tissues resulting in a potentially higher yield from the renewed bark. Thus, sustainable economic yield is achievable for a productive lifespan of 30 to 35 years from the LFLHS. These advantages of LFLHS contribute to reducing the tapper requirement and cost of production, ensuring increased profits and a longer economic lifespan of rubber production.

Keywords: Latex harvesting system • Tapping cost • Tapper productivity • Tapper requirement

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Zaw ZN^{1*}

¹ Agricultural Innovation and Management Division, Faculty of Natural Resources,
Prince of Songkla University, Songkhla, 90110, Thailand

*Correspondence: Email: spszarni@gmail.com

Introduction

Hevea brasiliensis is an indispensable economic crop supplying natural rubber commodity for the production of various rubber products. Over 85% of the global natural rubber demand is supplied by smallholders who own small-scale farms of less than ten hectares, and most rely solely on rubber production for their primary income (Association of Natural Rubber Producing Countries, 2021). Thus, the natural rubber industry is vital in supporting millions of farmers' livelihoods with a significant income source (Fox and Castella, 2013). With the economic growth and industrialization in rubber-producing countries, demand for labor has increased, resulting in a significant rise in general wages since the year 2010s (Ra, 2014; Ali et al., 2020). Meanwhile, many extended areas, planted in recent decades when the rubber price was attractive, have reached the mature stage, requiring a considerable number of tappers for harvesting. The issues mentioned above are leading to a shortage of skilled workers for tapping, resulting in increased tapping cost, which constitutes around 70% of the total production cost (Vijayakumar et al., 2003). The problems have been aggravated under prolonged unstable rubber prices, affecting employment stability, particularly in smallholders' rubber production (Rodrigo et al., 2011).

To solve these issues, studies have introduced and looked into using low-frequency latex harvesting systems (LFLHS). This system reduces the tapper requirement and tapping cost compared to conventional tapping system, while also increasing land and labor productivity. Ultimately, this helps ensure the long-term profitability and resilience of rubber production (Soumahin et al., 2010; Zaw et al., 2017; Sainoi et al., 2017a). This article discusses the issues encountered by the smallholders and elaborates on the competitive advantages of LFLHS compared to the conventional tapping systems based on previous research.

Latex Harvesting

To obtain rubber latex, the bark of a mature rubber tree is shaved in a process called latex harvesting or tapping. This removes the ends of the latex vessels clogged with coagulated latex

from the previous tapping. Once the tapping commences, fresh latex is released from the vessels and flows down into a container. Then, after a few hours, the latex vessels' ends are clogged again with the coagulated latex. There are different tapping systems adopted in an attempt to increase yield or improve profitability. A tapping system is considered ideal if it provides maximum yield at minimum tapping cost, while ensuring satisfactory tree growth, bark renewal, productive lifespan, and minimal occurrences of wounds on the tapping panels and physiological disorders (Vijayakumar et al., 2000).

When selecting tapping systems, it is important to consider multiple factors, including the cultivar planted, tree age, number of tappable trees, weather conditions, availability of skilled tappers, rubber prices, and wage agreements to ensure the optimal results. The number of tapped trees, the height, length, direction, and slope of the tapping cut, the frequency and time of tapping, panel changing, bark consumption, and yield stimulation are basic technical elements to assess the tapping quality (Malaysian Rubber Board, 2009).

Conventional Latex Harvesting Practices

Typically, latex harvesting commences six or seven years after planting to ensure economic yield. It is considered to begin a plot for tapping when at least 50 or 70% of the total trees reach 45-50 cm circumference at 150 or 170 cm height above the ground. The opening of tapping is carried out in a downward direction at 150 cm height from the ground with a 30 to 35 degree angle from high left to low right. A tapping system traditionally recommended in most rubber-producing countries is a downward tapping of a half spiral tapping cut length (S/2) with alternate daily tapping (one day tapping followed by a tapping rest day – d2) without any yield stimulation (Rodrigo et al., 2011). The trees being tapped are split into two plots, with each plot being tapped alternatively. This allows a tapper to cover two plots efficiently. However, some countries, where smallholders are the majority of the production share and their farms are not an economically manageable size, are practicing high-frequency tapping systems, such as daily tapping (d1), four-day tapplings in five

days (4d5), three-day tappings in four days (3d4), and two-day tappings in three days (2d3) (Chantuma et al., 2011). In addition, erratic weather, like a prolonged heavy rainy season that disturbs the regular tapping works, leads the high-frequency tapping systems after the rainy season to compensate for the tapping days lost. These tapping systems require a higher number of tappers for a certain number of tapped trees and less tapper productivity, resulting in a significant higher tapping cost (Zaw et al., 2017).

Mechanism of Latex Flow

Fresh *Hevea* latex in the latex vessels mainly consists of rubber globules, lutoid particles, and Frey-Wyssling particles by dispersing with other constituents – amino acids, inorganic acids, proteins, carbohydrates, resins, glucosides, tannins, and alkaloids. Mineral salts, proteins, and sucrose are also soluble in parenchyma and phloem cells beside the latex vessels (Gomez and Hamzah, 1989; Bottier, 2020).

Before tapping, latex vessels are under high hydrostatic pressure. Meanwhile, osmotic pressure from surrounding cells also makes the hydrostatic pressure higher. This causes an increase in phloem turgor pressure in the vessels. Generally, hydrostatic pressure in the latex vessels before tapping in the morning ranges between 10 and 15 atmospheres while the ambient pressure is low. When the vessel is tapped, the latex is released due to a high-pressure difference (An et al., 2014). Then, a fall in pressure in the latex vessels to the ambient follows, and consequently, it allows water from the surrounding tissues to flow into the latex vessels, causing the latex less viscous and an enhanced flowing of latex (Vijayakumar et al., 2000; Yeang, 2005). After a certain duration, latex flow slows down, and cessation of the flow follows. It is because of an inherent clotting mechanism in the latex vessels. While latex flows out, lutoid particles are ruptured and release destabilizing substances called hevein, a kind of protein, which flocculates and coagulates the latex near the cut ends in the vessels resulting in clogging the latex flow (Shi et al., 2016; Sainoi et al., 2017b). Tree assimilation, transport of sugars, and sink capacity are primary factors influencing latex regeneration

(Silpi et al., 2007). The latex regeneration between the two tappings is related to the cellular metabolism of the laticifer system and physiological functioning of the tree (Chao et al., 2015). The complete regeneration of the latex in the vessels after one tapping was estimated to be around 48 to 72 hours, depending on the clonal latex metabolism capacity (Chantuma et al., 2022).

Yield Stimulation

The most common latex yield stimulant widely used in rubber production is 2-chloroethyl phosphonic acid which decomposes in the bark to release ethylene when applied, and extends the duration of latex flow by delaying the plugging of latex vessels (Zhu and Zhang, 2009). Due to the longer duration of latex flow, the amount of latex discharged increases during tapping. As latex production is associated with genetic features, environmental effects, and tapping systems, response to stimulation strongly depends on these different factors (Njukeng et al., 2011; Traore et al., 2011). Thus, stimulation must be applied cautiously by considering the yield potential of clonal typology with age, weather conditions, and tapping intensity.

Stimulation is an excellent means of removing limiting factors on latex flow. However, excessive use or misuse can lead to serious malfunctioning of the laticifers. Intensive stimulation causes an excessive outflow of latex, disorders the physiological state of the tree, and leads to degeneration of the laticiferous system in the bark (Jacob et al., 1989). In conventional tapping system like d2 frequency, yield stimulation is not recommended.

Low Frequency Latex Harvesting System

Implementing LFLHS means a reduction of tapping frequency which increases the number of days between two successive tappings, notably the latex regeneration period, resulting in higher yield per tree per tap and tapper productivity (Obouayeba et al., 2010; Karunaichamy et al., 2012). For conventional tapping with d2 frequency, the trees are split into two plots and tapped alternatively, with one plot being tapped each day. When tapping in d3

frequency (tapping every three days), the trees are divided into three plots, and each plot is then tapped once every three days.

Tapper Requirement. The LFLHS taps fewer trees per day in a certain productive area compared to the conventional system. This does not mean that the number of trees tapped by a tapper, known as the task size, is reduced. Under LFLHS, tapper is assigned to tap other tasks in the following days while the first task is resting, so that the trees of a certain task get more resting days for latex regeneration. Thus, LFLHS enables a tapper to cover more tasks. For instance, with the d3 frequency tapping system, one tapper can handle three tasks, while the d6 frequency tapping system allows one tapper to handle six tasks (Figure 1A). It highlights that the frequency of tapping plays a crucial role in determining the number of trees tapped per day and the number of tappers required (Zaw et al., 2017).

By switching from d2 to d3 tapping frequency, the tapper requirement can be reduced by 33% by increasing the number of tasks per tapper from two to three (Figure 1B). Similarly, under a d4 frequency, there is a 50% reduction in tapper requirement by increasing the land-man ratio by 50%.

Latex Production. Since LFLHS has a longer interval between two successive tapplings to allow for regeneration of replenishing laticiferous content, which was removed from previous tapping, a reduction in tapping frequency generally produces a higher yield than normal tapping frequency. The yield per tree per tapping (g/t/t) for d3, d4, and d6 are 30 to 100% higher than the d2 frequency tapping (Kewi and Sivakumaran, 1994; Karunaichamy et al., 2012). Besides, the yield under LFLHS throughout the year was stable and increased in production trend (Leilani et al., 2015). A study of Jacob et al. (1989) observed that although LFLHS resulted in a high yield per tapping per tree (high tapper productivity), the interval between the two tapplings longer than seven days led to a low yield significantly comparing between around 50 g/t/t under d4 frequency and around 30 g/t/t under d14 frequency tapping. The study noticed that a resting period of more than one week between two tapplings did not result in a significantly higher yield due to a notable decrease in metabolic activity.

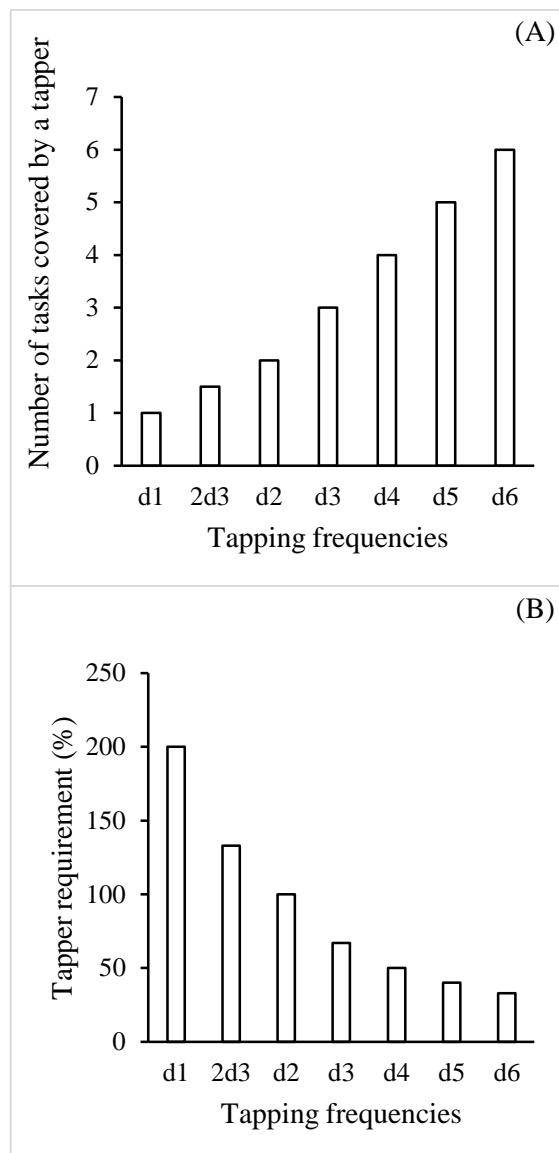


Figure 1. Number of tasks covered by a tapper (A) and tapper requirement in different latex tapping frequencies (B)

In a study of Thanh et al. (1996), cumulative yield (kg per tree per year) under d3 and d4 frequency tapping were respectively 93% and 86% of that obtained from the d2 tapping system. Nugawela et al. (2000) and Zaw et al. (2017) also found that cumulative yield from d3 frequency tapping was comparable and slightly higher than that of d2 frequency, while it was only 84-86% of 2d3 frequency tapping yield. Some studies suggested that it was economically profitable when the cumulative yield of LFLHS reached 90% of that of d2 frequency tapping, which was considered a break-even yield (Nayagam et al., 1986). Optimum land

productivity could not be achieved without combination of the yield stimulation under LFLHS (Sivakumaran et al., 2002; Lacote et al., 2013). The main objective of stimulation in LFLHS is to compensate the yield loss caused by the fewer number of tapped trees and tapping days (Rodrigo, 2007). It is important to adjust the stimulation's frequency and concentration based on tree's physiological status associated to weather, clones, tree age, and tapping system (Lacote et al., 2013).

Tapping Cost and Tapper Income.

Tapping cost is mainly associated with tapping frequency, tapper productivity, and payment system. LFLHS reduces the cost of tapping per unit production because of its low tapper requirement and high tapper productivity. With a certain higher productivity of the d3 frequency tapping system, the tapping cost could be reduced by about 20% from that of the d2 frequency tapping system (Nugawela et al., 2000). It was observed that the cost did not decrease under the product sharing payment system as it depended solely on rubber price (Zaw et al., 2017). However, the increased tapper productivity had a positive impact not only on the tapper income but also on the overall income of the farm in LFLHS. Tapper income is a crucial factor in addressing the skilled tapper shortage problem. Thus, some percentage of the benefits can be shared with skilled tappers as an incentive. In some estates, based on an over-targeted-yield-incentive system, tappers' income was attractive under LFHS as their daily productions (tappers' productivities) were higher than the normal targeted yield.

Bark Consumption and Productive Lifespan. Bark consumption is the thickness of bark shaved by tapping, depending on the tapper's skill and tapping frequency. The shaving should be thick enough to remove all plugged vessel ends for an optimum yield. In LFLHS, it needs to remove a slightly thicker bark shaving per tapping (Lacote et al., 2004). Table 1 shows the standard bark consumptions by different latex harvesting frequency. The monthly bark consumption in the d3 frequency was 15 to 40% less than the conventional high-frequency tapping systems. Rodrigo (2012) reported that although bark shaving per tapping in LFLHS was thicker than that of the conventional tapping, S/2, the effect was marginal compared to the overall bark saving per year by less frequency of tapping.

Table 1. Bark consumptions per tap and per month under different latex harvesting frequencies.

Latex harvesting frequency	Bark consumption per tap (mm)	Bark consumption per month (mm)
2d3	1.0-1.2	20-25 (125-133)
d2	1.0-1.2	15-20 (100)
d3	1.3-1.5	13-17 (85-86)
d4	1.5-1.7	12-15 (75-80)
d5	1.7-1.9	10-13 (65-67)
d6	1.8-2.0	9-11 (55-60)

Note: Figures in parenthesis represent percentage of d2 frequency.

With a significant decrease in bark consumption, the LFLHS is expected to have a longer economic lifespan. By using the S/2 d3 tapping system, the productive lifespan of the trees could be extended by at least four to eight years compared to the conventional S/2 d2 tapping system (Nugawela et al., 2000; Vijayakumar et al., 2003).

Table 2 shows the comparison of tapping years on the basal panel, at which tapping is started from 150 cm height from the ground, by different tapping systems. When comparing the tapping years of the S/2 d2 tapping system on virgin bark of basal panel, the tapping years of d3, d4, d5, and d6 frequency tapping systems can be extended from 4 to 10 years while high-frequency harvesting systems S/2 2d3 and S/3 2d3 can tap only 60% and 90% of the S/2 d2 tapping years, respectively. The slower rate of bark consumption gives enough time for the bark tissues to regenerate. This means that renewed bark could potentially produce a higher yield. As a result, the sustainable economic yield under LFLHS is expected for around 30-35 years.

Table 2. Tapping years on virgin bark of basal panels under different latex harvesting systems

Latex harvesting system	Tapping years on virgin bark of first basal panel	Expected tapping years on virgin bark of basal panels
S/2 2d3	3	6
S/3 2d3	3	9
S/2 d2	5	10
S/2 d3	7	14
S/2 d4	8	16
S/2 d5	10	20
S/2 d6	10	20

Conclusion

This review highlights the performance of LFLHS that reduces the tapper requirement without compromising the yield level, compared to the conventional tapping practice. In addition, it suggests with technical evidence that LFLHS can lead to sustainable economic yield over the productive lifespan. Thus, shifting from conventional harvesting systems to LFLHS is recommended as a sustainable manner to lessen the current problems of skilled tapper shortage and increased production cost, ensuring a longer economic lifespan of rubber production.

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Amien S · Darmawan NP · Fathya D

Induction of ploidy level on three patchouli cultivars by colchicine *in vitro*

Abstract. Patchouli plants produce essential oils and are used as a raw material and fixative agent for perfumes. The most widely cultivated patchouli plant in Indonesia is Aceh patchouli. Sexual propagation in Aceh patchouli plants is impossible as it does not flower. Genetic diversity can be elevated through another method, such as polyploidy induction using colchicine. This research aimed to evaluate the state-of-the-art application of polyploidization techniques as a breeding tool for callus-based explants. A completely randomized design with a factorial pattern was used in this experiment consisting of two factors, patchouli cultivars (Sidikalang, Tapak Tuan, and Lhokseumawe) and colchicine concentration (0.0%, 0.2%, 0.5%, 0.7%, and 1%). The result showed an interaction between three patchouli cultivars with colchicine concentration on callus size and color characters. In Sidikalang cultivar, 0.2% colchicine concentration affects the character of callus emergence time and has more buds. In cultivar Tapak Tuan, the application of colchicine 0.2% affects the callus size character and has the highest number of buds. In the Lhokseumawe cultivar, giving 0.5% colchicine affects the character of callus appearance time, callus size, and callus color. Concentrations of 0.2% and 1.0% colchicine in Sidikalang and Tapak Tuan calluses increased the number of chromosomes, ranging from 2n (32), 3n (48), and 4n (64). The implication of the research could be disclosure of an opportunity to create a new superior variety.

Keywords: Aceh Patchouli, Breeding, Callus, Colchicine, *In Vitro*.

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Amien S^{1*} · Darmawan NP² · Fathya D²

¹ Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang Km. 21, Jatinangor, Sumedang 45363, Indonesia

² Undergraduate Programme of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang Km. 21, Jatinangor, Sumedang 45363, Indonesia

*Correspondence: suseno@unpad.ac.id

Introduction

Polyploidy is the possession of three or more complete sets of chromosomes. Generating synthetic polyploids as a plant breeding strategy has enabled the development of new and improved cultivars. Patchouli plants (*Pogostemon*) produce essential oils and are used as a raw material and fixative agent for perfumes. Lee & Lee (2019) have been reported that *Pogostemon* can prevent obesity, ameliorate inflammation in various cell types (Kim et al., 2021), ameliorates skeletal muscle insulin resistance and mediated suppression of inflammation (Pyun et al., 2021). In addition, patchouli oil is widely used in the cosmetic, antiseptic, and insecticide industries.

The most widely cultivated patchouli plant in Indonesia is Aceh patchouli (*Pogostemon cablin* Benth), with three superior cultivars, Tapak Tuan, Lhokseumawe, and Sidikalang. The development of new superior varieties will support the development of Patchouli alcohol-based products in Indonesia. Sexual propagation in Aceh patchouli plants is impossible as it has no flower.

Artificial polyploidization has increasingly become a prominent strategy in plant mutation breeding as it involves mutation of the genome as compared to other major types of mutation breeding for example gene mutation. Mutation that involves the genome will result in greater phenotypes variation as compared to gene mutation (Eng & Ho, 2019). It was also shown that polyploidization can increase the content of secondary metabolites and enlarge plant parts (Samatadze et al., 2022).

Colchicine is mutagen that is used both for polyploidy induction in plants and mutation induction. Colchicine, as a bioactive alkaloid and a poisonous compound, is extracted from seeds and corms of the meadow saffron (*Colchicum autumnal* L.) (Sattler et al., 2016). The main mechanism of colchicine is binding with alpha- and beta-tubulin dimers, which inhibits microtubule polymerization during the cell cycle (mitosis) in plant cells, after which chromosomes/chromatids migration is halted during the anaphase stage. It is acknowledged that cell division is blocked by colchicine mutagen, but its accurate mechanism in chromosomes and polyploidy induction of plants is still uncertain (Manzoor et al., 2019).

According to Eigisti & Dustin (1957) colchicine can work effectively at concentrations

of 0.01-1%, but each plant species may give different responses. The effect of colchicine on the growth of explants-based callus from three cultivars of patchouli is not yet known, so further research is needed. This research aimed to evaluate the state-of-the-art application of polyploidization techniques as a breeding tool for callus-based explants.

Materials and Method

This experiment was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Padjadjaran from April to October 2019. Shoot tips as an explant was existed from the patchouli plantlets of Sidikalang, Tapak Tuan, and Lhokseumawe cultivars, from the collection of Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Padjadjaran. Murashige & Skoog (1962) medium was used in this experiment include sterile distilled water (aquades), colchicine, methanol, kinetin 100 ppm, NAA (Naphthaleneacetic acid) 100 ppm, HCl 1N, and NaOH 1N.

A Complete Randomized Design (CRD) with factorial pattern consisting of two factors was applied in this experiment. The first factor were the patchouli cultivars (Sidikalang, Tapak Tuan, and Lhokseumawe), and the second factor consisted of five levels of colchicine concentration (0.0% (as control), 0.2%, 0.5%, 0.7%, and 1%). Each treatment was replicated three times, with 3 units of culture bottles per treatment, resulting in 90 culture bottles. To determine if there are significant differences among the treatments, a 5% level F-test was conducted to test the effect of each treatment. If the 5% level F-test results show significant differences, a Scott-Knott test is used for further comparison.

The experiment started by sterilizing the equipment, making media and colchicine solution, soaking explants in colchicine, planting explants, maintaining culture, observing cultures, and conducting a cytology test. The tissue culture equipment and culture bottles were sterilized in an oven at a temperature of 150°C for 1 hour. The laminar airflow hood (LAF) was sterilized using 70% alcohol. Subsequently, the growth medium was prepared, and the colchicine solution was prepared. The culture bottles containing the sterilized medium were autoclaved at a temperature of 121°C and a pressure of 1 atm. The explants were immersed in colchicine solutions with concentrations of 0%, 0.2%, 0.5%, 0.7%, and 1%

for 30 minutes. The explants from the immersion were planted in culture bottles containing MS medium + 2 ppm kinetin + 0.2 ppm NAA. The response and growth of the explants were observed from the beginning of the planting until the end of the experiment. Cytological tests were performed to count the number of chromosomes generated from the experiment.

Observations were made on the time of callus formation, callus size, color, time of shoot formation, and number of shoots. Time of callus formation observation was conducted when any callus appeared on the explants, and it was counted from the beginning of planting. The callus size was measured using a clay model scale. Callus color scoring was based on the Munsell Color Chart for Plant Tissue. The time of shoot formation was counted from the time of planting until the end of the experiment. The number of shoots was determined by counting the newly emerged shoots. Plantlet sample chromosome was observed using Carton microscope (Japan).

Result and Discussion

The results of the F test on the main observations (Table 1) show that there is an interaction between cultivars and colchicine concentrations in the callus size and callus color characters. Interactions that were not significantly different between cultivars and colchicine concentrations were found in the number of shoots and the time of callus appearance. Significant differences in colchicine concentrations were shown for all characters. Further analysis was carried out on data that showed significant differences using the Scott Knott advanced test.

Table 1. Calculated F values for the main observation characters.

Factor B (Colchicine)	Factor A (Cultivar)		
	v ₁	v ₂	v ₃
k ₀	5.00 a	5.00 a	5.00 a
k ₁	7.33 b	5.67 a	5.00 a
k ₂	7.00 b	6.33 b	6.67 b
k ₃	7.00 b	7.00 b	7.00 b
k ₄	7.00 b	7.00 b	7.00 b

Note : * significantly different at the 5% level
tn is not significantly different at the 5% level

Time of callus formation. Callus is an unorganized tumor tissue that emerges as a result of injury to an organ or differentiating tissue (Joya et al., 2020). In this study, callus formation

occurred at the lower part of the explants (Figure 1), specifically at the site of the previous wound.



Figure 1. Callus Formed on Wounded Part

In Table 2, the fastest callus appearance time in the Sidikalang cultivar is observed in the control treatment (k₀), which is 5 days after treatment (DAT). The longest time is observed in the 0.2% treatment, with a time of 7.33 DAT. In the Sidikalang cultivar, the control treatment differs significantly from all the colchicine treatments (0.2%, 0.5%, 0.7%, and 1%), while all the colchicine treatments have the same notation.

Table 2. The independent test results from the response of three Patchouli cultivars to colchicine concentration on time of callus formation.

Variance	Main Observation			
	Time of Callus Appearance	Callus Size	Callus Color	Number of Shoots
Cultivar	1.04 ^{tn}	17.00*	11.18*	1.23 ^{tn}
Colchicine Concentration	5.32*	12.29*	28.82*	2.27 ^{tn}
Interaction	0.7 ^{tn}	3.33*	2.89*	1.34 ^{tn}

Note : v₁ = Sidikalang variety, v₂ = Tapak Tuan variety, v₃ = Lhokseumawe variety; k₀ = control, k₁ = colchicine 0.2%, k₂ = colchicine 0.5 %, k₃ = colchicine 0.7%, k₄ = colchicine 1%

The control treatment in the Tapak Tuan and Lhokseumawe cultivars also gave the fastest callus formation time of 5 DAT. There are no significant differences between the 0.2% colchicine treatment and the control treatment in both cultivars. The slowest callus appearance time is observed in the Lhokseumawe and Tapak Tuan cultivars in the 0.7% and 1% colchicine treatments, which is 7 DAT. The fastest callus appearance time is observed in the control treatment in all three cultivars. The longest time is observed in the Sidikalang cultivar with the 0.2% colchicine treatment. The range of callus appearance time in all treatments are between 5-7.33 DAT. The delay in callus appearance time in

the explants treated with colchicine can be attributed to the inhibition of cell division. Colchicine is a compound that acts as a mitosis inhibitor. The application of colchicine at the plant's growing point prevents the formation of spindle fibers and the separation of chromosomes during anaphase of mitosis resulting in slower cell division (Zhou et al., 2017).

Callus size. The callus that appears on the explants is caused by cells in contact with the medium being stimulated to become meristematic and subsequently undergo active division, similar to wound-covering tissue (Ikeuchi et al., 2017). The formed callus continues to divide, leading to an increase in size. In this experiment, the measurement of callus size was conducted weekly using a clay model scale.

Table 3. The effect of cultivar and colchicine concentration interaction on the callus size character.

Treatment	Mean	Notation
v ₃ k ₁	12.63	A
v ₃ k ₀	12.63	A
v ₂ k ₀	12.21	A
v ₃ k ₂	11.54	B
v ₂ k ₁	11.17	B
v ₁ k ₀	10.67	C
v ₃ k ₃	10.46	C
v ₁ k ₂	10.17	C
v ₁ k ₃	10.13	C
v ₂ k ₃	9.75	C
v ₂ k ₂	9.75	C
v ₁ k ₁	9.67	C
v ₃ k ₄	9.34	C
v ₁ k ₄	9.21	C
v ₂ k ₄	8.84	C

Note : v₁k₀= Sidikalang control; v₁k₁= Sidikalang with 0.2% colchicine; v₁k₂= Sidikalang with 0.5% colchicine; v₁k₃= Sidikalang with 0.7% colchicine; v₁k₄=Sidikalang with 1% colchicine; v₂k₀= Tapak Tuan control; v₂k₁= Tapak Tuan with 0.2% colchicine; v₂k₂= Tapak Tuan with 0.5% colchicine; v₂k₃= Tapak Tuan with 0.7% colchicine; v₂k₄= Tapak Tuan with 1% colchicine; v₃k₀= Lhokseumawe control; v₃k₁= Lhokseumawe with 0.2% colchicine; v₃k₂= Lhokseumawe with 0.5% colchicine; v₃k₃= Lhokseumawe with 0.7% colchicine; v₃k₄= Lhokseumawe with 1% colchicine.

According to the test results, there is an interaction between cultivar and colchicine concentration. The largest callus size is observed in the control treatment (v₃k₀) and the 0.2% colchicine treatment (v₃k₁) for the Lhokseumawe cultivar, with a size of 12.63 (A). Meanwhile, the smallest callus size is obtained in the Tapak Tuan cultivar with the 1% colchicine treatment, with a

size of 8.84 (C) (Table 3). These result in line to a study conducted by (Luo et al., 2018), in *Taraxacum kok-saghyz* seedlings treated with 0.1% colchicine resulted in tetraploid plants. In *Allium ascalonicum* plants, polyploidy induction with maximum 1% colchicine resulted in variations in form, size, and chromosome number (Wen et al., 2022).

In the Sidikalang cultivar, the largest callus size is observed in the control treatment (k₀), which is 10.67. The smallest callus size is found in the 1% colchicine treatment (k₄), with a size of 9.21. In this cultivar, the 0.2%, 0.5%, 0.7%, and 1% colchicine treatments do not show significant differences compared to the control treatment.

The largest callus size in the Tapak Tuan cultivar is observed in the control treatment, which is 12.21 (A). The smallest callus size is shown in the 1% colchicine treatment, which is 8.84 (B). The 0.2% colchicine treatment (k₁) in this cultivar shows a smaller size compared to the control treatment, but it has a significantly larger size compared to the other colchicine treatments (k₂, k₃, and k₄).

In the Lhokseumawe cultivar, the largest callus size is observed in the control treatment (k₀) and the 0.2% colchicine treatment (k₁), which is 12.63 (A). The smallest callus size in this cultivar is observed in the 1% colchicine treatment, with a size of 9.34 (C). An example of the comparison of callus sizes can be seen in Figure 2.

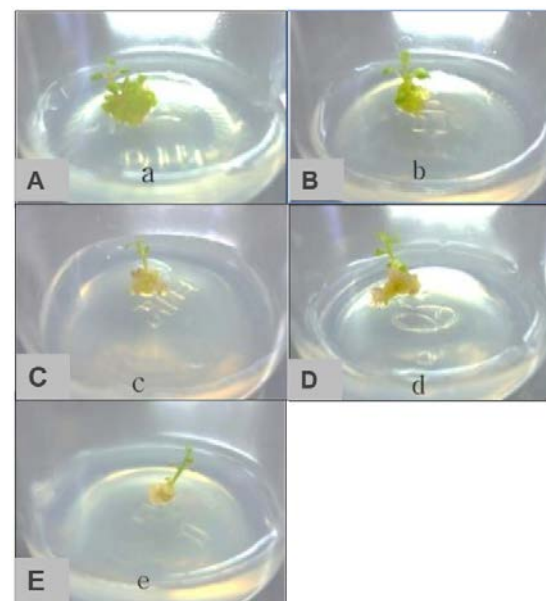


Figure 2. Callus size comparison for Lhokseumawe cultivar on 2 Weeks After Treatment (WAT)

Note: a. control; b. colchicine 0,2%; c. colchicine 0,5%; d. colchicine 0,7%; e. colchicine 1%.

Figure 3 showed that the growth of callus size in all treatments tends to increase every week. In explants treated with colchicine, the callus that appears continues to experience a significant increase until 8 WAT. This is in accordance with research conducted by Wibisono et al. (2022) that the application of colchicine can stimulate callus formation in *Plectranthus amboinicus* (L.). The growth of callus size in the control treatment in each cultivar in week 6 and week 7 began to slow. In the control treatment, the callus began to regenerate into shoots so that callus growth became slow.

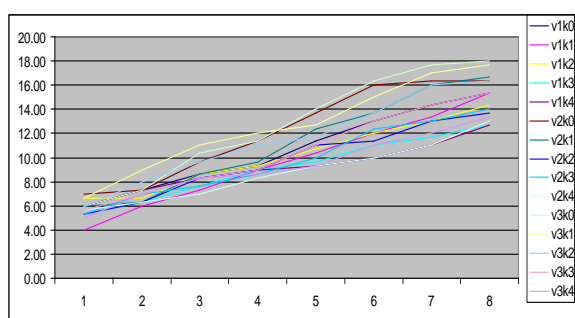


Figure 3. Callus Size Growth Chart

Callus color. The in vitro growth response of plants in each genotype will vary. Besides being influenced by genotypes, differences in response can also be affected by media and certain special treatments. In this experiment, the media was added with plant growth regulators in the form of cytokinin and auxin. The addition of cytokinin can increase chlorophyll content. Cytokinin inhibits the breakdown of chlorophyll and protein grains to inhibits the breakdown of chlorophyll and protein grains to slow down the process of senescence (aging) of cells (Sosnowski et al., 2023).

The color of the callus resulted in this experiment varied. Callus color was calculated using callus color parameters and calculated by scoring. Examples of callus color scoring on patchouli plant explants can be seen in Figure 4. The higher the concentration of colchicine, the callus produced tends to be more brownish (Figure 4). The color of the callus produced in the 1% colchicine treatment for all cultivars has a lower score compared to the control treatment. This is thought to be caused by colchicine residues still present in the explants that inhibit cell division and chlorophyll formation.

Figure 4. Examples of Callus Color Scoring on Patchouli

Note: A. Score 1 in Sidikalang cultivar with 1% colchicine (v_1k_4); B. Score 2 in Sidikalang cultivar with 0.7% colchicine (v_1k_3); C. Score 3 in Lhokseumawe cultivar with 0.5% colchicine (v_3k_2); D. Score 4 in Tapak Tuan cultivar with 0.5% colchicine (v_2k_2); E. Score 5 in Lhokseumawe cultivar with control treatment (v_3k_0).

In this experiment, the brownish-white callus was shown by the control treatment of Sidikalang cultivar with a score of 2.04 (Table 4). Green callus was obtained from the Lhokseumawe cultivar control treatment with a score of 4.38. This treatment was not significantly different from the control treatment of the other two cultivars (Tapak Tuan and Lhokseumawe).

Table 4. The effect of cultivar and colchicine interaction on the callus color character

Treatment	Mean	Notation
v_3k_0	4.38	A
v_2k_0	4.29	A
v_2k_1	4.21	A
v_1k_0	4.21	A
v_2k_2	3.96	A
v_1k_1	3.96	A
v_3k_1	3.88	A
v_2k_3	3.38	B
v_3k_3	3.29	B
v_3k_4	2.79	C
v_3k_2	2.71	C
v_2k_4	2.46	C
v_1k_2	2.25	C
v_1k_3	2.09	C
v_1k_4	2.04	C

Note : v_1k_0 = Sidikalang control; v_1k_1 = Sidikalang with 0.2% colchicine; v_1k_2 = Sidikalang with 0.5% colchicine; v_1k_3 = Sidikalang with 0.7% colchicine; v_1k_4 = Sidikalang with 1% colchicine; v_2k_0 = Tapak Tuan control; v_2k_1 = Tapak Tuan with 0.2% colchicine; v_2k_2 = Tapak Tuan with 0.5% colchicine; v_2k_3 = Tapak Tuan with 0.7% colchicine; v_2k_4 = Tapak Tuan with 1% colchicine; v_3k_0 = Lhokseumawe control; v_3k_1 = Lhokseumawe with 0.2% colchicine; v_3k_2 = Lhokseumawe with 0.5% colchicine; v_3k_3 = Lhokseumawe with 0.7% colchicine; v_3k_4 = Lhokseumawe with 1% colchicine

According to Faramayuda et al., (2022), the callus's green color indicates chlorophyll's presence. In this experiment, Callus that has a green color can regenerate into new shoots. This indicates that green callus can regenerate and divide actively in addition to containing a lot of chlorophyll.

Time of shoot formation. Shoot formation in tissue culture can occur in two ways, which are direct and indirect morphology (through the callus phase). In this experiment, the explants experienced indirect shoot formation. Not all callus in the treatment successfully formed buds.

The initial time of shoot emergence shows how fast the explants respond to the treatment in producing shoots. All cultivars' fastest shoot emergence time was 25 days (Table 5). In the Sidikalang cultivar (v_1), no shoots were formed at colchicine concentrations of 0.5%, 0.7%, and 1%. While in Tapak Tuan (v_2) cultivar, buds were not included at 1% colchicine concentration. In the Lhokseumawe cultivar, the higher colchicine concentration resulted in a longer time for buds to appear.

Table 5. Patchouli shoot emergence time.

Treatment		Replication		
		I	II	III
v ₁	k ₀	25	T	25
	k ₁	35	35	32
	k ₂	T	T	T
	k ₃	T	T	T
	k ₄	T	T	T
	k ₀	25	25	32
v ₂	k ₁	40	T	T
	k ₂	T	35	T
	k ₃	47	40	40
	k ₄	T	T	T
	k ₀	32	25	32
	v ₃	k ₁	T	32
k ₂		T	35	T
k ₃		T	56	T
k ₄		T	56	56

Note: v_1 = Sidikalang variety, v_2 = Tapak Tuan variety, v_3 = Lhokseumawe variety; k_0 = control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%, T= No shoot formed

In 0.2% and 0.5% colchicine treatment, Tapak Tuan and Lhokseumawe cultivars showed the appearance of buds. However, until the end of the observation, the shoots failed to formed. At week 9 to week 13 after treatment, both the shoots and callus in all experimental units experienced browning. This is thought to be caused by the reduced amount of nutrients that plants from the media can absorb. Thus, inhibiting the growth of

callus and shoots. Morphological comparison of callus, budding, and patchouli shoots can be seen in Figure 5.

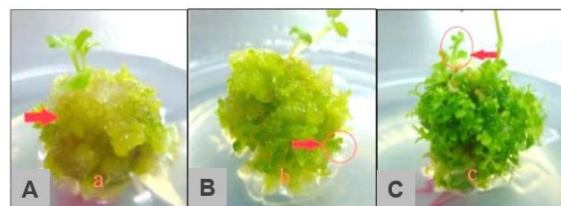


Figure 5. Morphological comparison of callus, budding, and patchouli shoots on Lhokseumawe Cultivar: a. Callus in 0.7% colchicine (k_3), b. Budding in 0.5% colchicine (k_2), dan c. Shoot in control treatment (k_0).

Number of shoots. In this experiment, the number of shoots was counted to determine the effect of colchicine on callus regeneration and shoot multiplication. The number of shoots is often related to the success of tissue culture multiplication. The more the number of shoots formed, the more multiplication can be done to get more new shoots. The number of shoots is known by counting new shoots that have a height of + 0.5 cm.

Table 6. Results of independent test response of three Patchouli cultivars to colchicine concentration on number of buds character

Factor B (colchicine)	Factor A (cultivar)		
	v_1	v_2	v_3
k_0	1.67 a	16.67 a	4.00 a
k_1	3.67 a	0.67 b	1.67 a
k_2	0.00 a	0.67 b	1.00 a
k_3	0.00 a	1.67 b	0.33 a
k_4	0.00 a	0.00 b	0.67 a

Note : v_1 = Sidikalang variety, v_2 = Tapak Tuan variety, v_3 = Lhokseumawe variety; k_0 = control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%

From the data in Table 6, the best number of shoots on Sidikalang cultivar was shown by 0.2% colchicine treatment. Meanwhile, the colchicine treatment of 0.5% (k_2); 0.7% (k_3); and 1% (k_4) did not grow shoots. In Lhokseumawe cultivar, the highest number of shoots was shown by the control treatment (k_0) which was as many as 4. The least number of shoots in this cultivar was obtained from 0.7% colchicine treatment with a total of 0.33. In this cultivar, an increase in colchicine concentration tends to reduce the

number of buds that can be produced. According to Sinta & Widoretno (2020), using the same growth regulator, an increase in colchicine concentration can reduce the increase in the number of shoots of Crown Vetiver Plant (*Vetiveria zizanioides* L. Nash)

After performing independent tests on cultivars Sidikalang and Lhokseumawe, colchicine treatment at each concentration level was not significantly different from the control treatment. This is following the opinion of Wibisono et al. (2022) that some colchicine treatments on two species *Mischanthus* not significantly different from the control treatment on the character of the number of shoots and the percentage of callus regeneration.

In Tapak Tuan cultivar, the highest number of shoots was produced from the control treatment, that is 16.67 (Table 6). Meanwhile, this cultivar's 1% colchicine treatment (k₄) did not grow shoots. Based on the results of independent tests, the control treatment is significantly different from all levels of colchicine treatment. In colchicine treatment 0.2% (k₁); 0.5% (k₂); 0.7% (k₃); and 1% (k₄) were not significantly different from each other. This indicates that the increase in colchicine concentration does not statistically affect the number of shoots character. The application of colchicine during in vitro micropropagation is only sometimes associated with low regeneration of treated parts.

Cytology test. At week 13, subcultures were conducted on experimental units that were still green or not browning. Subculture is an attempt to replace tissue culture growing media with new media, so that nutritional needs for growth can be met. After subculturing, the growth process is shown by the increase in callus size and the formation of shoots from the buds.

Subculture in this experiment was also carried out as an effort to form roots. The formed roots will be used as material in the cytology test. According to Saensouk and Saensouk (2021), roots are suitable explants for chromosome tests. The cytology test was conducted to determine the extent to which colchicine can affect the number of chromosomes of the experimental unit.

In Lhokseumawe cultivar, the control treatment (k₀) showed the fastest time with 2 weeks after subculture. Meanwhile, the colchicine treatments of 0.2% (k₁), 0.5% (k₂), and 1% (k₄) did not grow roots. The fastest root emergence time in Tapak Tuan cultivar was shown by the control treatment (k₀) and 0.2%

colchicine treatment (k₁) which was 2 weeks after subculture. The other colchicine treatments (0.5%; 0.7%; and 1%) did not grow shoots. The fastest root emergence time in Sidikalang cultivar was 3 weeks after subculture. In all colchicine treatments (0.2%; 0.5%; 0.7%; and 1%) did not grow shoots (Table 7).

Table 7. Patchouli Root emergence time.

Factor B (Colchicine)	Factor A (Cultivar)		
	v ₁	v ₂	v ₃
k ₀	3	2	2
k ₁	T	2	T
k ₂	B	T	T
k ₃	B	T	3
k ₄	T	T	T

Note: v₁ = Sidikalang variety, v₂ = Tapak Tuan variety, v₃ = Lhokseumawe variety; k₀ = control, k₁ = colchicine 0.2%, k₂ = colchicine 0.5 %, k₃ = colchicine 0.7%, k₄ = colchicine 1%, T = No roots formed ; B = browning

Table 8. Number of Shoot after Subculture

Treatment		Repeat			Average
		I	II	III	
v ₁	k ₀	70	B	90	80
	k ₁	B	B	6	6
	k ₂	B	B	B	B
	k ₃	B	B	B	B
	k ₄	21	40	B	30.5
v ₂	k ₀	70	170	K	120
	k ₁	B	16	30	23
	k ₂	14	20	50	42
	k ₃	B	38	B	38
	k ₄	B	B	9	9
v ₃	k ₀	33	60	16	3.86
	k ₁	8	B	B	8
	k ₂	16	20	41	25.6
	k ₃	85	23	65	57.6
	k ₄	40	B	B	40

Note: v₁ = Sidikalang variety, v₂ = Tapak Tuan variety, v₃ = Lhokseumawe variety; k₀ = control, k₁ = colchicine 0.2%, k₂ = colchicine 0.5 %, k₃ = colchicine 0.7%, k₄ = colchicine 1%, K = No shoot formed ; B = browning

At week 4 after subculture, samples were taken for cytology test. The samples used were Tapak Tuan cultivar with 0.2% colchicine and control (v₂k₁ and v₂k₀). Subcultures performed on several experimental units showed success in shoot multiplication. This is indicated by the increasing number of shoots produced. The highest number of shoots was obtained from Tapak Tuan cultivar in the control (v₂k₀) replicate II with 170 shoots (Table 8). The least number of buds obtained from the Sidikalang cultivar treated with colchicine 0.2% (k₁) in a total of 6. Transferring the plants to new media during

subculture made the nutrients needed by the plants for the re-growth process available, so that regeneration or bud formation could also be carried out.

Morphological diversity in the treated plantlets was seen in morphological observations in the 9th week after subculture. The Tapak Tuan cultivar that was treated with 0.7% colchicine (v_2k_3) had larger leaves compared to the control treatment (v_2k_0) (Figure 6).

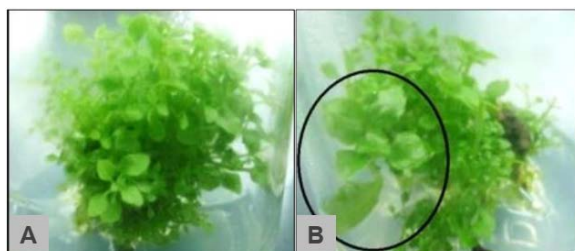


Figure 6. Morphological diversity in Tapak Tuan Cultivar (v_2) : A. Control Treatment (k_0) and B. Colchicine Treatment 0.7% (k_3). Black circle shows bigger leaf size

Diversity was also seen in the Sidikalang cultivar treated with 1% colchicine (v_1k_4) which had larger leaves and fewer shoots than the control treatment (v_1k_0) (Figure 7). This is in accordance with research conducted by Sinta & Widoretno (2020) on patchouli plants those treated with colchicine had greener shoots, sturdier stems and wider leaves compared to the control plants. Colchicine treatment on *Platycodon grandiflorus* plants can also produce mutants that have thicker and wider leaf morphology compared to control plants (Wu et al., 2011). Treatment with colchicine was thought to have succeeded in inducing polyploidy, so that the resulting plantlets had larger sizes than the control plants. Some characteristics of polyploid plants according to Manzoor et al. (2019) are having a larger size and slower cell division than diploid plants.

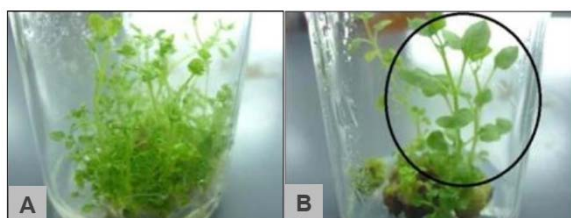


Figure 7. Morphological diversity in Sidikalang Cultivar (v_2) : A. Control Treatment (k_0) and B. Colchicine Treatment 1% (k_4). Black circle shows bigger leaf size

Cytological test was conducted to determine whether colchicine can affect the number of chromosomes. The samples plantlets for cytology tests were taken in the 4th week after subculture. The sample used is the treatment that has the fastest root emergence time and has a diversity of growth during the observation. The sample plantlets used for this cytology test were v_2k_0 and v_2k_1 . In Figure 8, it can be seen that the v_2k_0 plant has sufficient roots many emerge from the stems and the undersides of shoots. The buds produced by v_2k_1 appear to have a different color from the buds from v_2k_0 . The color of the v_2k_1 buds tends to be greener than the v_2k_0 buds.

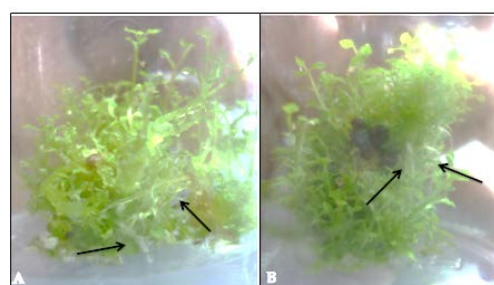


Figure 8. Roots formed on Tapak Tuan Cultivar A. Control Treatment (k_0); B. colchicine 0.2% (k_1).

Cytological test results on sample plants showed that there were differences in the number of chromosomes in the control Tapak Tuan cultivar (v_2k_0) and Tapak Tuan cultivar treated with 0.2% colchicine (v_2k_1). The v_2k_0 plant has 32 chromosomes (Figure 9), while the v_2k_1 plant has 48 chromosomes (Figure 10). According to Oyen (1999), Aceh patchouli's normal number of chromosomes is 32. This shows that the number of chromosomes in the control treatment (v_2k_0) is the same as the average number of chromosomes for Aceh patchouli and the number of chromosomes in the v_2k_1 treatment has an additional number of chromosomes of 48 or triploid.

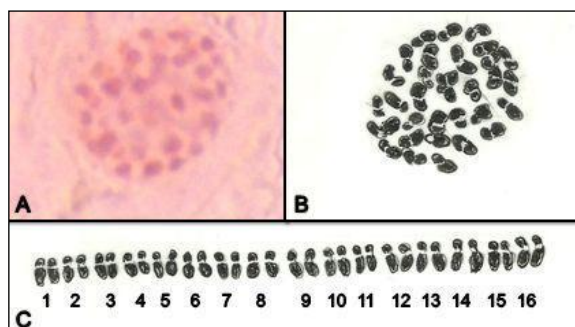


Figure 9. Chromosome number of Tapak Tuan Cultivar with control treatment (v_2k_0) : A. Microscopic figure of the test result, B. Number of chromosome $2n=32$, C. Caryotype

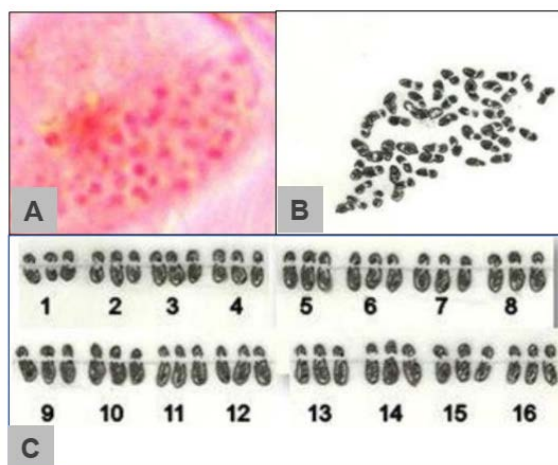


Figure 10. Chromosome Number of Tapak Tuan cultivar treated with Colchicine 0,2% (v₂k₁): A. Microscopic figure of the test result, B. Number of chromosome 2n=48, C. Caryotype

Colchicine works by thwarting the formation of spindle fibers and causing the chromosomes to spread across the equator without moving toward the poles. When a critical colchicine concentration is maintained in the cell, chromosome doubling can occur repeatedly. Still, if colchicine is only given for a short time, the long fibers (spindle fibers) can reform, and the polyploid cells can produce nuclei of their own (Samatadze et al., 2022). When soaking shoots of Sidikalang cultivar (v₂) with 0.2% colchicine (k₂) in this experiment, it is possible that the spindle fibers failed to form and caused an increase in the number of chromosomes. The polyploid cells formed in the experimental unit succeeded in multiplying and regenerating into new plants.

Table 9. Polyploidy level on cultivar promoted by colchicine.

No.	Treatment	Number of Planlet	Number of Chromosome (2n)
1.	v ₁ k ₀	3	32 (3)
2.	v ₂ k ₀	4	32 (4)
3.	v ₃ k ₀	3	32 (3)
4.	v ₁ k ₄	6	32 (1), 48 (2), 64 (3)
5.	v ₂ k ₁	16	32 (6), 48 (5), 64 (1)
6.	v ₃ k ₄	2	64 (2)

Note: v₁ = Sidikalang variety, v₂ = Tapak Tuan variety, v₃ = Lhokseumawe variety; k₀ = control, k₁ = colchicine 0,2%, k₂ = colchicine 0,5 %, k₄ = colchicine 1%

Testing the number of chromosomes in the plantlet root samples from each cultivar showed variations in the number of chromosomes.

Colchicine concentrations of 0.2% and 1.0% increased polyploidy levels varying from diploid (2n=32), triploid (3n=48), and tetraploid (4n=64) to Tapak Tuan and Sidikalang cultivars (Table 9).

Varied level of polyploidy can be affected by kind of explant, cultivar, culture period, basic medium especially plant growth regulator and concentration of Colchicine.

Ploidization opens up possibilities for the development of new superior varieties of aromatic and medicinal plants such as Patchouli. Iannicelli et al. (2020) have reviewed the advantages of the ploidization method. Ploidization has different advantages compared to other methods in plant breeding, including on the genetic characteristics, biochemical and phenotypical.

Conclusion

1. There is an interaction between three patchouli cultivars with colchicine concentration on callus size and callus color characters.
2. In Sidikalang cultivar, 0.2% colchicine concentration affects the character of callus emergence time and has more buds. In Tapak Tuan cultivar, the administration of colchicine 0.2% affects the callus size character and has the highest number of buds. In Lhokseumawe cultivar, giving 0.5% colchicine gives a real effect on the character of callus appearance time, callus size, and callus color.
3. Colchicine 0,2% and 1% could promote varied level of polyploidy of planlet-derived callus on Tapak Tuan and Lhokseumawe Cultivar.

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