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PREFACE

Jurnal Kultivasi started a new volume in 2023, published as Vol 22 No 1 with the full English version. This section contains 14 articles on agriculture topics such as agronomy or plant production, plant breeding, soil science, and protection plant science. An expert editor and reviewer with advanced expertise have already reviewed the published article in this number. The editor team was supported by Indonesia Scientists and an overseas team qualified with the related knowledge to enrich our journal to expand internationally. On the other hand, Kultivasi also invited the foreign reviewer to encourage this section to be more acceptable to the whole world. We select the manuscript that submits to our journal more competitively based on the criterion of the deep enlightenment and novelty of the article information.

This journal also has re-new the template and guidelines that should be concerned by the author when submitting the article for the next edition. We modify this journal for maintaining the journal value and to reach the author and readers from other countries. Furthermore, Kultivasi already got re-accreditation status and settled in Rank 2 on the National accreditation journal from 2022 until 2027 based on the Ministry of Education, Culture, Research, and Technology of Indonesia pronouncement on 30 December 2022. This achievement will inspire Kultivasi Team to improve every edition and preserve the quality of the published research articles. Thank you very much to the author, editor, reviewer, and especially the reader who always supports us to improve every time.

Editor

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 suplementary 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 peel 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 writen 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).
- Three or more authors: Nurmala et al. (2014) or (Nurmala et al., 2014).
- 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.
  Example: Solaimalai A, Anantharaju P, Irulandi S, Theradimani M. 2021. Maize Crop Improvement, Production, and Post Harvest Technology. CRC Press. Oxon.
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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.

CONTENTS

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Noli ZA · Suwirmen · Julita

Effect of *Padina minor* powder extract as biostimulant and black soldier fly fertilizer on growth and yield of soybean (*Glycine max* L. Merrill)

Abstract. Soybean production has not met domestic demand, causing dependence on imported soybeans. Increasing soybean production can be done by giving organic materials such as fertilizer and biostimulants. Applying *Padina minor* extract as a biostimulant and black soldier fly (BSF) fertilizer can be an alternative to increase the growth and production of soybean. The research aims to determine the effect of *Padina minor* powder extract, BSF fertilizer, and the combination of *Padina minor* powder extract and BSF fertilizer on the growth and yield of soybean. This research was carried out from April to July 2021 at the Plant Physiology Laboratory and Greenhouse, Department of Biology, Andalas University, Padang. The experiment was arranged in a Completely Randomized Design with four treatments (control or without extract and fertilizer), *Padina minor* extract, BSF fertilizer, and the combination of *Padina minor* extract and BSF fertilizer) and six replications. The results showed that the extract of *Padina minor* did not significantly affect the growth and yield of soybean. BSF fertilizer significantly increased the number of leaves, branches, leaf area, chlorophyll b, and total chlorophyll of soybean. The combination of *Padina minor* extract and BSF fertilizer gave similar effects as BSF fertilizer on increasing the number of leaves, leaf area, chlorophyll b, and total chlorophyll of soybean.

Keywords: Biostimulant · Black Soldier Fly · Organic fertilizer · *Padina minor*

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Introduction

Soybean is the third strategic food crop commodity after rice and corn, making them a target commodity in food self-sufficiency (Handriawan et al., 2016). Soybean consumption and demand increase along with population growth, but domestic soybean production have yet to meet the increasing demand for soybeans. It causes domestic soybean needs to be met from imports (Nuhung, 2013). Over the last decade, soybean imports reached 67% of the national soybean demand, indicating that domestic production can only meet 33% of the demand (Swastika, 2015). Soybean imports until 2020 have reached 2.48 million tons (Statistics Indonesia, 2020), while MOA estimates that soybean imports in 2021 will reach 2.6 million tons. Therefore, increasing the growth and production of soybean in Indonesia needs to be done using biostimulants and organic fertilizers.

Biostimulants are formulations of bioactive compounds applied to plants to improve nutrient absorption efficiency, abiotic stress tolerance, and plant quality (Calvo et al., 2014; Du Jardin, 2015). Biostimulants can provide nutrients, increase availability (Kesaulya et al., 2015), and modify plant physiology processes such as respiration, photosynthesis, nucleic acid synthesis, and ion absorption (Abbas, 2013). Sources of biostimulants include microbial inoculum, humic acid, fulvic acid, amino acids, seaweed extracts, and plant extracts (Calvo et al., 2014).

Seaweed can be used as a biostimulant because of the high diversity of seaweed in Indonesia and its suboptimal use. The seaweed in Indonesia reaches 12 million hectares but has only been utilized around 281,474 ha (Ministry of Marine Affairs and Fisheries, 2019). The use of seaweed extract as a biostimulant has been widely studied and proven to affect the growth and development of roots and shoots, photosynthesis, increase plant vigor and delay fruit aging (Zodape et al., 2011; Pise & Sabale, 2010). Hadi et al. (2016) reported that there are five types of seaweed in Nirwana Beach, Padang, West Sumatra, that have the potential to be used as biostimulants. Noli et al. (2021; 2022) reported the screening results of seaweed from West Sumatra and showed that *Padina minor* extract gave the best results in spurring germination and vegetative growth of soybeans.

In addition to seaweed, using organic matter as a booster for plant growth and development can be obtained through the bioconversion of organic waste by bioconversion agents (bacteria, fungi, and insect larvae). One of the insect larvae used as a bioconversion agent is the *Hermetia illucens* or Black Soldier Fly (BSF) species (Kinasih et al., 2018), which is commonly found in palm oil waste. The *Hermetia illucens* larvae are known as maggot.

BSF larvae or maggots have been widely used as bioconversion agents. They can overcome the problem of organic waste because of their ability to reduce 50-80% of organic waste per day from the amount of food they get (Balitbangtan, 2016; Diener et al., 2011). In addition, BSF larvae can process organic matter into products that can be used as fertilizer. Yuwono and Mentari (2018) reported that the analysis of organic waste content decomposed by BSF larvae showed that it could be declared as compost and relatively well function like compost within 30 days of the composting process. Another study by Nirmala et al. (2020) reported that the results of waste decomposition derived from 100% vegetables, 100% fruits, and a mixture of 80% vegetables + 20% fruits aged 15 days met the requirements of good compost.

Reswita et al. (2021) reported that maggot bioconversion fertilizer could improve the physical and chemical properties of Ultisol soil and increase the grain weight of 100 upland rice seeds. Pratama (2020) reported that solid fertilizer from former BSF larvae could increase stem height, root length, number of leaves, and leaf area of chili at a ratio of 1:3 for BSF fertilizer and soil. The concentration ratio has the highest average value compared to compost and NPK fertilizer.

In this study, the effect of *Padina minor* extract as a biostimulant and Black Soldier Fly fertilizer was tested to increase the growth and yield of soybean. This study aims to determine the effect of *Padina minor* extract, BSF fertilizer, and the combination of both on the growth and yield of soybean (*Glycine max*).

Materials and Methods

This study was conducted from April 2021 to July 2021 at the Plant Physiology Laboratory and Greenhouse, Department of Biology, Andalas University, Padang. The tools used in this study

were polybags (60 x 40 cm), collection plastic, a grinder, analytical scales, measuring cups, sprayers, filter paper, label paper, stationery, cameras, meters, shakers, centrifuge, and spectrophotometers. The materials used in this study were *Padina minor* seaweed, BSF fertilizer, soybean seeds (Anjasmoro var.), aquadest, 80% acetone, Ultisol soil, manure, Urea fertilizer, KCl fertilizer, and TSP fertilizer.

This experiment used a Completely Randomized Design with four treatments such as control (without the application of extract and fertilizer), *Padina minor* extract (25 mL), BSF fertilizer (300 g), and the combination of *Padina minor* extract and BSF fertilizer, with six replications for each treatment.

Fertilizer obtained from Maggot Farming Business in Mungka, 50 Kota Regency, a place for waste management and BSF maggot cultivation. Nutrient analysis has been carried out, and it is known that BSF fertilizer has the main nutrient content, where the concentration of N is 3.219%, P is 1.705%, K is 0.534%, and C/N content is 6.445%. *Padina minor* collected at Nirwana Beach, Padang, West Sumatra. *P. minor* cleaned of sand and mud attached to seawater, then put it in a labeled plastic bag. The collected *P. minor* was then rewashed with tap water to remove any remaining salt and sand, air-dried the samples for four days, then pulverized to obtain a coarse powder. The coarse powder was then weighed and soaked with hot water in a ratio of 1:20 (w/v) for sample and water, stirred for 24 hours at room temperature then filtered. Dissolved the resulting filtrate in 1 liter of water and put it into a storage bottle (Norra et al., 2016).

The extract was sprayed for 25 ml when the soybeans were 2, 4, and 5 weeks old after planting (Kalaivanan et al., 2012; Grabowska et al., 2012; Zakiah et al., 2017). BSF fertilizer was applied for 300 g at the beginning of seed planting and when soybeans were four weeks after planting (WAP).

Soybean seeds were planted in a mixture of Ultisol soil and manure (5:1), as much as 10 kg/polybag. Maintenance included watering, weeding, and fertilizing. Fertilizer application was carried out based on the recommended dosage for soybean, which was 50 kg/ha for urea fertilizer, 100 kg/ha for TSP, and 100 kg/ha for KCl or equivalent to 0.45 g Urea, 0.9 g TSP and 0.9 g KCl. Half of Urea dosage was applied at the beginning of seed planting and when the plant was 30 days old, while 1 part of KCl and TSP was applicated at the beginning of seed planting (Rukmana & Yudirachman, 2014; Zakiah et al., 2017).

The parameters observed were height, number of leaves, number of branches, leaf area, wet weight, dry weight, leaf chlorophyll content, number of pods, the weight of the entire seed, and weight of 100 seeds per plant. The data were tested with Analysis of Variance (ANOVA) and continued with the Duncan New Multiple Range Test (DNMRT) at 5%.

Results and Discussion

The results of the vegetative parameters analysis of soybean are presented in Table 1. Based on the table, it is known that *Padina minor* extract alone had not been able to provide a significant effect on all vegetative growth parameters of soybean; however, BSF fertilizer provides a significant effect in increasing the parameters of the number of leaves, the number of branches and leaf area. A combination of *P. minor* and BSF fertilizer also significantly influenced the parameters of leaf number and leaf area. It is shown that applying BSF fertilizer was an effective treatment to increase the growth of the number of leaves, leaf area, and the number of branches of soybean.

The vegetative growth of plants is affected by the nutrients absorbed. In this case, the macro-nutrient nitrogen (N) contained in BSF fertilizer is thought to play a role in increasing the number and area of leaves and branches on soybean plants. Nitrogen is needed for the vegetative growth of plants, mainly stems, branches, and leaves, and is a constituent component of amino acids, proteins, and the formation of cell protoplasm, which can stimulate plant growth (Safei et al., 2014; Lingga and Marsono, 2013). The results of Zahn's research (2017) showed that applying 5 g of BSF fertilizer (kasgot) increased *Allium cepa*.

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Table 1. Number of leaves, number of branches, leaf area, height, wet weight and dry weight of soybeans with the application of *P. minor* extract and BSF fertilizer

Treatment	Number of Leaves (strands)	Number of Branches	Leaf Area (cm^2)	Height (cm)	Wet Weight (g)	Dry Weight (g)
Control	50.33 a	5.33 a	53.51 a	53.04 a	133.63 a	37.89 a
P. minor extract	58.66 a	5.66 a	49.48 a	53.58 a	150.83 a	38.35 a
BSF fertilizer	70.40 b	7.00 b	76.09 b	54.11 a	158.36 a	39.58 a
P. minor extract + BSF fertilizer	74.40 b	6.00 ab	85.62 b	54.98 a	167.90 a	40.59 a

Note: Numbers 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 %.

Plant height did not show significant results with the application of BSF fertilizer, allegedly because BSF fertilizer plays more role in vegetative growth processes that lead to the increasing growth of leaves and branches. The significant results on the number and area of leaves and the number of branches were in line with the weight of seed parameter (Table 3), which showed that the application of BSF fertilizer tends to increase the weight of soybean seeds. The number of branches and leaves correlated with the increase in photosynthetic product, which will also enhance the metabolism of plants to grow and produce properly (Syaifudin et al., 2019; Ermawati et al., 2018). A large number of branches tends to be followed by increased pods, resulting in more seed yield (Dwiputra et al., 2015).

A combination of *P. minor* extract and BSF fertilizer also showed significant results on the number of leaves and leaf area. However, giving *P. minor* extract alone did not significantly affect all growth parameters. It showed that BSF fertilizer was more dominant in affecting the improvement of some growth parameters of soybean than *P. minor* extract. It is shown that *P. minor* extract alone could not increase photosynthetic and other metabolic activities, leading to the increase of various plant metabolites responsible for cell division and elongation (Kanwal et al., 2016). It did not have a significant effect on the vegetative growth of soybean plants.

The effect of *P. minor* extract, BSF fertilizer, and the combination of both on chlorophyll levels of soybean plants is presented in Table 2. Based on the table, it is known that chlorophyll b and total chlorophyll of soybean leaves showed markedly different results against the application of BSF fertilizer and a combination of extracts and fertilizers. While the application of *P. minor* extract did not differ markedly on chlorophyll a, chlorophyll b, and total chlorophyll levels of soybean plants showed that *P. minor* extract exerted the same effect as BSF fertilizer and did not differ markedly from controls. This showed that BSF fertilizer and the combination of fertilizer with *P. minor* extract could influence the increase in chlorophyll levels of soybean plants, *and P. minor* extract had not been able to exert a significant influence in increasing chlorophyll levels of soybean plants on Ultisol soils.

Table 2. Chlorophyll levels of soybean plants treated with *P. minor* extract and BSF fertilizer

Treatment	Chloro-phyll a (mg/g)	Chloro-phyll b (mg/g)	Total Chlorophyll (mg/g)
Control	0.5138 a	0.9348 a	1.4487 a
P. minor extract	0.5458 a	1.0398 ab	1.5858 ab
BSF Fertilizer	0.5578 a	1.1257 b	1.6837 b
P. minor extract+BSF fertilizer	0.5615 a	1.0694 b	1.6311 b

Note: Numbers 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 %.

BSF fertilizer and the combination of *P. minor* extracts and fertilizers were able to affect the chlorophyll levels of soybean plants allegedly because of the availability of Mg nutrients in fertilizers which were known to play an essential role in the process of chlorophyll formation in plants, where Mg acts as the central atom of the chlorophyll molecule (Lingga and Marsono, 2013). Mg deficiency in plants will interfere with electron transport in

photosynthesis, resulting in a decrease in chlorophyll content and CO2 fixation and impaired carbon metabolism (Farhat et al., 2014). Research by Wu et al. (2019) reported that BSF organic fertilizer significantly increased chlorophyll levels in tomato plants. Similar results were reported by Reswita et al. (2022), that upland rice plants treated with BSF bioconversion fertilizer had higher chlorophyll levels.

Table 2 shows that the application of *P. minor* extract did not show statistically significant differences in the chlorophyll content of soybean plants. However, there was a tendency for higher chlorophyll levels in applying *P. minor* extract compared to the control. This indicates that *P. minor* extract could increase plants' chlorophyll levels. Similar results were reported by Noli et al. (2021), where the application of *P. minor* seaweed extract had no effect statistically on soybean plants' chlorophyll levels but tended to increase in frequency treatment and different applications when compared with controls.

Table 3. Number of pods, weight of seeds and weight of 100 seeds of soybean plants treated with *P. minor* extract and BSF fertilizer

Treatment	Number of Pods	Weight of Seeds (g)	Weight of 100 Seeds (g)
Control	96.33 a	52.71 a	29.35 a
P.minor extract	91.33 a	58.22 ab	32.51 a
BSF fertilizer	101.93 a	60.99 b	30.08 a
P.minor extract+ BSF fertilizer	104.76 a	63.24 b	30.39 a

Note: Numbers 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 result showed in Table 3 that the treatment of BSF fertilizer and the combination of *P. minor* extract with BSF fertilizer had a significantly different effect than *P. minor* extract on the weight of seeds of the soybean plant. Meanwhile, the application of *P. minor* extract alone had not been able to exert a markedly different effect on all generative parameters of soybean plants compared to the control treatment. It showed that BSF fertilizer could increase soybean yield on the weight of seeds parameter. BSF fertilizer provided sufficient nutrients for plants that had a role in seed synthesis and other metabolism resulting in the increase of seed weight in soybean plants. Dinariani et al. (2014) stated that organic fertilizers would be well decomposed during tillage, so that plant roots quickly absorb nutrients in the soil.

Phosphorus (P) and potassium (K) contained in BSF fertilizer had 1.705% for P content and 0.534% for K content. These two elements had an essential role in the formation of soybean seeds. The effect of BSF fertilizer on crop production was reported by Tanga et al. (2021); the combination of BSF larvae fertilizer and NPK in corn (*Zea mays*) produced grains 23-68% higher than other fertilizer treatments, and BSF fertilizer alone provided higher grain yields than crops given commercial organic fertilizer (Evergrow). Research by Menino et al. (2021) added that BSF fertilizer significantly affected the overall production of ryegrass (*Lolium multiflorum*).

Providing sufficient P nutrients that plants can absorb will increase the weight of soybean seeds. P nutrients play a role in plant cell division, strengthening rooting and accelerating the flowering and ripening of seeds (Supartha et al., 2012). Hayati et al. (2012) added that the benefits of P fertilizer are to support the beginning of root growth, the growth of flowers and seeds, and the increase in the percentage of flower formation into seeds. Potassium is an activator of many enzymes that are important for the photosynthesis process; it also helps form starch and protein, so it plays an essential role in increasing the number of pods and seeds in plants. Potassium has a low ion exchange capacity and is often replaced by aluminum ions in acidic soils, so potassium ions can potentially be lost (Subandi, 2013; Puspitasari and Elfarisna, 2017).

Conclusion

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

1. *P. minor* powder extract did not affect the growth and production of soybean (*Glycine max*).
2. Black Soldier Fly fertilizer increased the number of leaves, branches, leaf area, chlorophyll b, and total soybean chlorophyll (*Glycine max*).

3. The combination of *P. minor* powder extract and black soldier fly fertilizer increased the number of leaves, leaf area, chlorophyll b, and total soybean chlorophyll (*Glycine max*).

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Total soluble solid and titratable acidity in different fruit maturation stages of *Solanum lycopersicum cv*. micro-tom and its mutant *iaa9-3* and *iaa9-5*

Abstract. Fruit development influences the metabolite contents and then its biological activity; however, such report is still limited in tomato *IAA9* mutants. This study aims to evaluate total soluble solid and titratable acidity in several stages of fruit maturation of the mutant micro-tom tomato. The experimental method used is the t-test method with three replications and followed by correlation and principal component analysis. The tested genotype were *iaa9-3* and *iaa9-5* mutants against *WT-MT*. Pearson correlation analysis showed that *iaa9-3* and *iaa9-5* produced higher levels of total soluble solid and titratable acidity in different fruit maturity levels; and the increase of flowering age and all fruit maturity ages, except for the breaker age that was similar to *WT-MT* tomato.

Keywords: Tomatoes · Fruit maturity level · *iaa9* · Fruit quality

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Introduction

Tomato *(Solanum lycopersicum)* provides a vitamin containing food, carotenoid and phenolic compounds (Marti et al., 2016). Growing environmental factors may affect the number, size and quality of fruit (Albert et al., 2016). The ambient growth of plants with high temperatures can lead to the failure of tomato formation (Hoshikawa, 2017). High temperature responses can be overcome by a tomato metabolic change (Albert et al., 2016).

Metabolic changes can be done with molecular technologies such as the use of gene mutations (Saito et al., 2011). Mutation in the 'Hong Anliu' orange bud can increase monosaccharides and but decrease the levels of organic acid (Pan et al., 2013). Arabidopsis of mutants *iaa-5, iaa-6,* and *iaa-19* planted at heat stress can reduce 45–50% of the primary root length and fresh weight, while control plants have dead plant conditions (Shani et al., 2017). Tomato var. *Ailsa Craig* granted for exogenous auxin at 10^{-5} is needed to delay maturing in mature green and ten days before the phase breaker while the red phase has increased its ripening rate (Cohen et al., 1996).

Fruit development is a multiphase process that requires close coordination between molecular, biochemical, and structural elements. DNA modification leads to metabolic properties resulting from the differences in metabolic pathways. A nutrient quality metabolic capacity results in differences in fruit growth and maturation (Zhang et al., 2010). Studies of the mutant effect in the *IAA9* genes for changes in metabolic properties and fruit maturity age in tomatoes have not been made, thus present study aimed to evaluate total soluble solid and titratable acidity in several stages of fruit maturation of the mutant micro-tom tomato.

Materials and Methods

Study Site. The study was conducted for three months in the hydroponic garden of Bale Tatanen Faculty of Agriculture, Universitas Padjadjaran, with an altitude of ± 750 meters above sea level. The post-harvest quality test was conducted for three months in the Horticulture Laboratory, Faculty of Agriculture Universitas Padjadjaran.

Materials. Plant materials used are the Micro-Tom (MT) tomato seeds, namely wild type of MT (WT-MT), *iaa9-3,* and *iaa9-5* mutants. Other materials were AB mix hydroponic nutrition, charcoal chaff, cocopeat, compost, and insecticide. The materials used in the tomato nutrition analysis are fresh tomato samples, NaOH, and aqua dest.

The items needed in the laboratory were analytical balances, pH meters, 100 mL and 50 mL cups, micro pipets, blender, aluminium foil, plastic wrap, spectrophotometer UV-vis Orion AquaMate 8000 (Thermo Scientific, USA), refractometer PAL-J (Atago, Tokyo, Japan), refrigerator, analytic scale, micro tube, mortars, and aquadest. The needed tools in the field were pots and watering cans.

Preparation of plant. Mutant Micro- Tom tomatoes namely *iaa9-3* and *iaa9-5*; and its *WT-MT were* tested by two testing stages of generic growth and fruit quality. The experimental method used was the t-test which was followed by a principal component analysis.

Generative Growth

The Flowering Age. Flowering age is measured by counting the number of days after seedling until the first flower in one flowering plant.

Figure 1. Fruit maturation stage of Micro Tom and its mutant

Fruit Maturation Stage. The fruit maturation stage is varied, i.e., the green, mature green, breaker, pink and red. Harvesting is done when the fruit meets the harvest criteria. The harvest criteria used in present experiment were as follows (Mubarok et al., 2019) :

- Green (G) (Flowering +20 day): The color of the fruit are showing green.
- Mature Green (MG) :
  The color of the fruit is bright green normally called mature green.
- Breaker (Br):
  The discolored condition of the fruit indicates fragmentation in green with yellow or pink at the base of the fruit about 10%.

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- Pink (P): The color of the fruit are showing pink with age (Br+3).
- Red (R): The color of the fruit indicating a deep red to the entire surface of the fruit (Br + 7).

Fruit Quality. The fruit quality test criteria consist of three phases of maturation: breaker, pink dan red.

- **Total Soluble Solid (TSS)**
  The mutant *iaa9-3* tomato and *iaa9-5* are prepared in microtubes with a weight of fruit juice of about 5 g. Microtube was then centrifuged at 1000 rpm. Supernatan 1000 rpm was collected using a micropipette and transferred to the refractometer lens (Majidi et al., 2011).
- **Titratable Acidity (TA)**
  Titratable acidity measurements are done using 5 mL of tomato juice that was titrated by NaOH 0,1 N (Tilahun, 2013).

Result and Discussions

The Flowering Age (DAP). The results of the flowering age analysis can be shown in Figure 2.

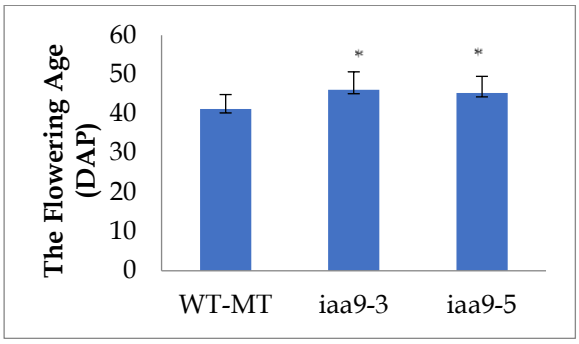

Figure 2. The flowering age of *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Mutants *iaa9-3* and *iaa9-5* had significantly slower flowering age than the *WT-MT* (Figure 2). Mutant *iaa9-3* and *iaa9-5* have a longer life span of 46 DAP and 45 DAP than the *WT-MT* of 41 DAP. A mutation effect on the *IAA9* gene can induce early ovarian growth before the flower blooms (Kim et al., 2020). The effects of mutations in the genes *IAA9*, causes cell expansion in the thickness of the ovarian wall (Kim et al., 2020). The thickening of the ovarian wall result in increased cell size, the number of cell layers and the area of mesocarp (Kim et al., 2020).

The success of cell division and expansion in flowers is the beginning of fruit growth (Gillaspy et al., 1993). Ovula embryo bags containing vascular ovaries and micropylar poles are where auxin accumulates (Pattison and Catala, 2012). Auxin was accumulated in ovula embryo six days before anthesis (Pattison and Catala, 2012). In auxin mutant eggplant *parental advice-1 pad-1*, IAA levels rise just when the flowers bloom, while the *WT-MT* decreases and remains low at the right time (Matsuo et al., 2020). The affects the rise in flower formation, the increase in the formation of fruits and the formation of partensian fruits, which results in much more desirable results (Matsuo et al., 2020).

The Age of Green Fruit Maturity Stage (DAP). Research indicates that the green phase age of the mutants was *iaa9-3* and *iaa9-5* significantly from statistics compared with the *WT-MT* (Figure 3). Mutants *iaa9-3* and *iaa9-5* had the slower green phases of 66 DAP and 65 DAP than *WT-MT* of 61 DAP (Figure 3). The slower life span resulting from a mutation influence in the *IAA9* gene (Figure 2), affected the green harvests lifespan compared with the *WT-MT* (Figure 3).

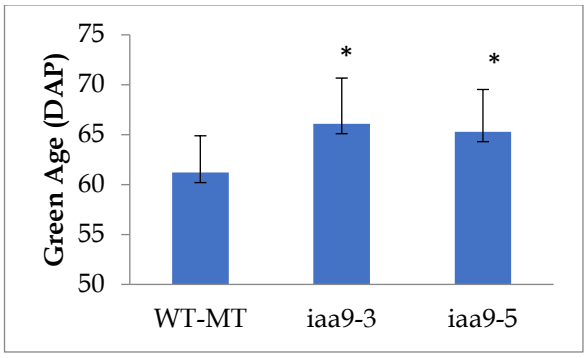

Figure 3. The age of green fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

The tomato is climacteric, which means that at the beginning of the fruit growth, the automatic de-blocking phase of "system 1" is called (Kumar et al., 2014). The ripening of the

fruit of the "system 1" has a reduced maturing response a level of accumulated basic ethic and ethylene sensitivity (Kumar et al., 2014). The maturation of "system 2" takes a place at the beginning of the auto catalitic phase (the rapid rise of ethylene) so that ethylene is called the fruit development transition (Giovannoni et al., 2021).

IAA can change regulation from system 1 to system 2 by pressing ethylene and abscisic acid (ABA) as a result of crosstalk auxin and GA while beginning fruit development (Pattison et al., 2015). The regulation of transportation for auxin on the maturation of the fruit can present a high degree of ethylene sensitivity (Shin et al., 2019). This led to the significant slower of green age of mutant *iaa9-3* and *iaa9-5* fruit than its WT-MT. This is supported in the study of *Ailsa Craig* tomatoes provided with auxin exogen 10^{-5} and a got a delay of maturation green stage and 10 days before phase breaker (Cohen et al., 1996).

The Age of Mature Green Fruit Maturity Stage (DAP). Research shows that the mature green age of mutant *iaa9-3* and *iaa9-5* is significantly different to *WT-MT*. Mutants *iaa9-3* and *iaa9-5* are slower mature green of 75 DAP and 74 DAP compared to the *WT-MT* of 70 DAP (Figure 4). The slower age of the green in the *IAA9* (Figure 3) gene mutation of the tomato, affected the lagging of the mature green phase fruit harvest compared with the *WT-MT*.

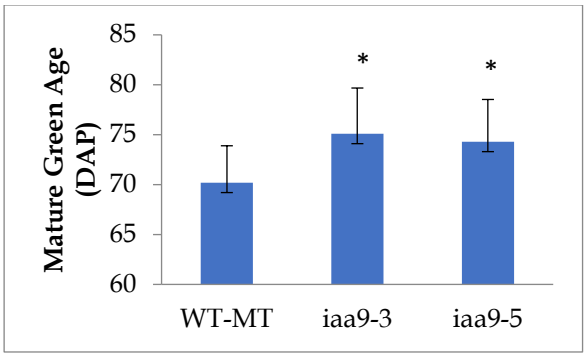

Figure 4. The age of mature green fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in $p < 0.05$

Auxin can control cell division and differentiate into stomata (Balcerowicz and Hoecker, 2014). Temperature 30-35 °C mutant *iaa9-5* and *iaa9-3* have a higher number of stomata of about 160 stomata mm^{2-} and 156 stomata mm^{2-}, respectively, compared with *WT-MT* of about 146 stomata mm^{2-} (Mubarok et al., 2020). The formation and distribution of higher stomata are affected by the auxin pathways (Balcerowicz dan Hoecker, 2014).

The increase in the number of stomata in the tomato plays a key role in improving photosynthesis, thus creating fruit growth (Wang et al., 2009). Increased photoactivity in plants can increase the accumulation of starch and the metabolism of sugars in the fruit (Pattison et al., 2015). Photosynthate is transported to the fruit in order to maximize the growth of the fruit of mature green phase (Wu and Kubota, 2008). The increased fruit reached its maximum size on the mature green stage and at the breaker stage the fruit size remained virtually unchanged (Wu and Kubota, 2008). The mutations in *iaa9-3* and *iaa9-5* have a distinct mature green age slower than the *WT-MT*.

The Age of Breaker Fruit Maturity Stage (DAP). Research shows that the age of breaker in mutants *iaa9-3* and *iaa9-5* are not significantly different than the *WT-MT*. Mutants *iaa9-3* and *iaa9-5* have a timeless age breaker which is 81 DAP and 80 DAP compared to the *WT-MT* which is 82 DAP (Figure 5). Auxin can control crucial processes in the development of the fruit (Teale et al., 2006). Auxin can encourage the initiation of fruit formation by stimulating the appearance of the hormone gibberellin (Serrani et al., 2008). The metabolism of GA can cause the parthenocarpy occurance in Arabidopsis of auxin mutants. Parthenocarpy occurs because pollen fails to fertilize ovula, producing several signals to encourage fruit initiation (Molesini et al., 2020).

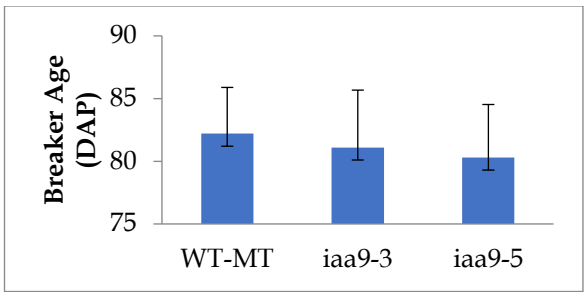

Figure 5. The age of breaker fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in $p < 0.05$

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The Age of Pink Fruit Maturity Stage (DAP) Research has shown that the pink phase ages on mutants *iaa9-3* and *iaa9-5* are not significantly different according to statistics compared with the *WT-MT*. Mutants *iaa9-3* and *iaa9-5* have a faster pink phase age and are not significantly 84 DAP and 83 DAP than *WT-MT* is 85 DAP. The increase in the number of chlorophyll can increase photosynthesis in plants, in effect can increase the accumulation of starch and metabolism in fruit (Pattison et al., 2015).

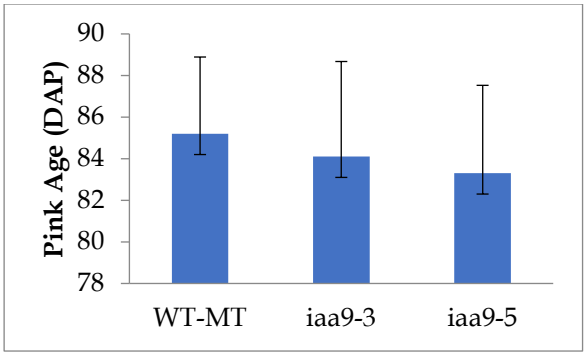

Figure 6. The age of pink fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

The Age of Red Fruit Maturity Stage (DAP). Research shows that the longevity of red phase in mutant *iaa9-3* and *iaa9-5* is not significantly different to *WT-MT* (Figure 7). Mutants *iaa9-3* and *iaa9-5* have a faster and surer years of the red phase, which is 88 DAP and 87 DAP compared to *WT-MT* which is 89 DAP.

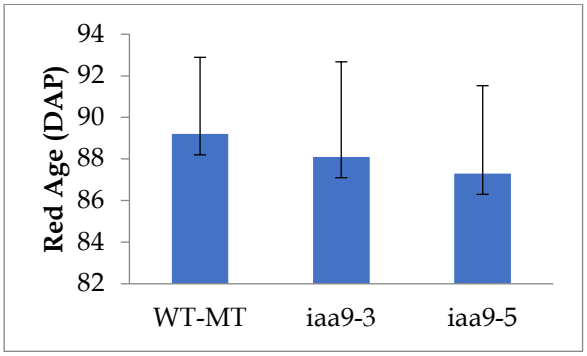

Figure 7. The age of red fruit maturity stage in *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Mutants *iaa9-3* and *iaa9-5* have a significant TSS and TA value higher on breaker, pink and red fruit maturity than the *WT-MT* (Figure 8 and Figure 9). This leads to the longevity of the mutants *iaa9-3* and *iaa9-5* at red maturity levels precisely despite the slowdown in the maturity stages of *green* dan *mature green*. The hormone auxin affects proper and efficient fruit growth and thus can coordinate normal tomato growth (Gorguet et al., 2005).

Total Soluble Solid (TSS). Mutants *iaa9-3* and *iaa9-5* have significantly higher TSS values in breaker, pink and red fruit maturity than in *WT-MT* (Figure 8). Mutants *iaa9-3* and *iaa9-5* at the breaker maturity age have higher TSS value of 4.933 and 5.133 °Brix compared to *WT-MT* which is 4.133° Brix (Figure 8). Mutants *iaa9-3* and *iaa9-5* with pink maturity has a higher TSS value of 6.467 and 6.767 °Brix than the *WT-MT* of 6.133 °Brix (Figure 8). Mutants *iaa9-3* and *iaa9-5* at red maturity level have a higher TSS rate of 6.767 and 7.2 °Brix compared to *WT-MT* which is 6.567 °Brix (Figure 8). High quality tomatoes score for TSS in breaker phase and red phase were breaker 4.47 °Brix and 6,57 °Brix, respectively (Campos et al., 2006).

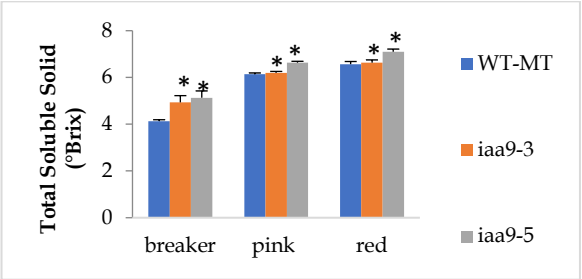

Figure 8. Total Soluble Solids of *iaa9-3*, *iaa9-5* and *WT-MT*
Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Auxin appears as a negative regulator of SlGLK2 which can boost cytokinin response (Quinet et al., 2019). The interaction of cytokinin with auxin can affect the allocation of root biomass, promoting cell growth and root growth (Sachs, 2005). In the tobacco plant increased partition resulting from changes the CWIN in root and leaf activity (Werner *et al* 2008). This enzyme regulates the flow of sucrose by controlling the apoplastic removal of the floem

(Roitsch et al., 2003). Induction CWIN at the root increases the strength of cytokinin to build sink activity (Roitsch et al., 2000).

IAA appears to induce the activities of sink (sucrose allocation) and CWIN on young leaves and roots (Roitsch et al., 2003) This enzyme regulate the transport of sucrose by regulating the apoplastic degradation of floem (Roitsch et al., 2003). Total soluble solids is increasing at amylase stimulation and can interfere the quality of fruit during ripening (Quinet et al., 2019).

Titratable Acidity (TA). Mutant *iaa9-3* and *iaa9-5* have significantly higher TA values in breaker, pink and red fruit maturity than the *WT-MT* (Figure 9). Mutants *iaa9-3* and *iaa9-5* with breaker maturity levels have a higher TA rate of 0.49 and 0.494% compared to *WT-MT* 0.321% (Figure 9). Mutant *iaa9-3* and *iaa9-5* with a pink maturity rate have a higher 0.619 and 0.625% compared with *WT-MT* 0.564% (Figure 9). Mutant *iaa9-3* and *iaa9-5* at red maturity rate have a higher value of TA 0.702 and 0.756% compared with *WT-MT* 0.612% (Figure 9).

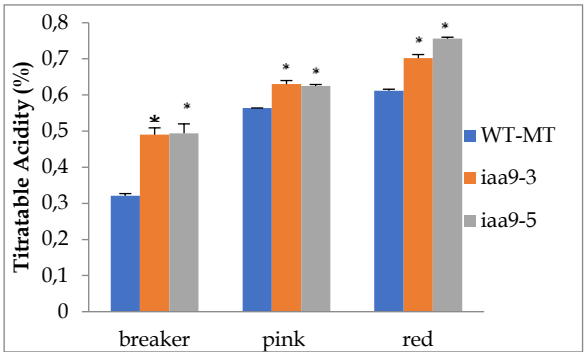

Figure 9. Titratable acidity of *iaa9-3*, *iaa9-5* and *WT-MT*

Average value ± Standard Error (SE) (n=30) followed by star (*) shows a significant different compared with control (*WT-MT*) according to Student's T-Test in p< 0.05

Previous styudy reported a positive correlated sugar content with titratable acidity (Georgelis, 2002). Positive correlation between sugar and titratable acidity suggests that plants with high sugar generally have more free organic acid and less concentration of hydrogen ions (Georgelis, 2002). The acidity of the higher fruit has the advantage of the yeast infection (Mohammed et al., 1999).

The Correlation of TSS, TA and the Ages of Fruit Maturation Stage in WTMT and its mutants

Table 1. Pearson correlation of TSS, TA and the Ages of Fruit Maturation Stage in WTMT and its mutants

Variable	B	G	MG	BR	P	R	TSS
G	1 *						
MG	1*	1*					
BR	(-) 0.83	(-) 0.83	(-) 0.83				
P	(-) 0.83	(-) 0.83	(-) 0.83	1*			
R	(-) 0.83	(-) 0.83	(-) 0.83	1*	1*		
TSS	0.63	0.63	0.63	(-) 0.9*	(-) 0.9*	(-) 0.9*	
TA	0.86	0.86	0.86	(-) 0.9*	(-) 0.9*	(-) 0.9*	0.9

Description: B- The flowering Age; G- green fruit maturity; MG- mature green fruit maturity; BR- breaker fruit maturity; P- pink fruit maturity; R- red fruit maturity; TSS- Total Soluble Solid; TA- Titratable Acidity

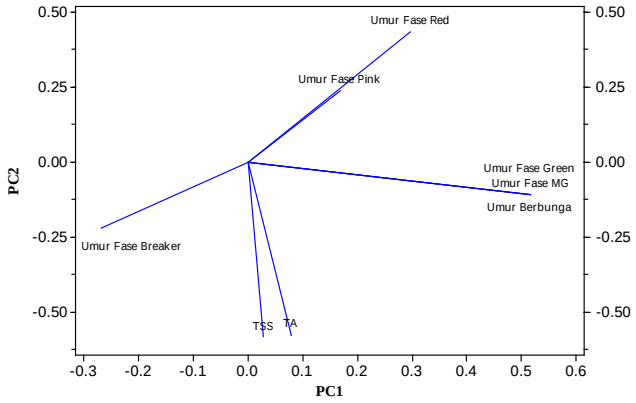

Figure 10. Biplot of the principal component analysis of total soluble solid, titratable acidity and age of fruit maturation stage

1. The biplot of principal component analysis is a 2-dimensional form of the combined main component 1 or PC1 and the main component 2 or PC2.
2. Biplot can explain 76,6% of the variety in the data population because it has a cumulative proportion of 0.766. This cumulative proportion was the accumulation of proportion on the PC1 of about 0.446 and PC2 of about 0.321.

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3. On PC1, the flower age, green age phase, mature green age phase, pink age phase, red age phase, TSS and TA has the same direction, while they had an opposite of the age of breaker maturation age (Figure 10). PC1 value is positive, except the age of breaker.

Conclusion

Mutation tomatoes in the *IAA9* genes produced an increased TSS and TA content at all fruit maturity levels, along with increased flowering age and all of the age of fruit maturity phases except the breaker that similar to WT-MT tomato.

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Effectiveness of water hyacinth compost and N, P, K, S fertilizer on S-available, S uptake, protein content, and yield of shallot in Inceptisols from Jatinangor

Abstract. Water hyacinth (*Eichhornia crassipes*) is a source of organic matter that can be used as compost to improve the soil quality and productivity of shallots. Shallots are horticultural commodities that have various benefits. Inceptisol soils dominate Indonesia, with an area of 37.5% of Indonesia's land area but have low soil fertility. Soil fertility can be increased by optimal fertilization. This experiment aimed to determine the dose of water hyacinth compost and nitrogen (N), phosphor (P), potassium (K), sulfur (S) fertilizer which gave the best effect on increasing available S, S uptake, protein content, and yield of shallots. The experiment was conducted from July to October 2021 at the Experimental Garden of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor. The experiment used a Randomized Block Design, consisting of seven treatments repeated four times. The recommended fertilizer doses used are 200 kg Urea, 500 kg ZA, 300 kg SP-36, and 200 kg KCl. The compost used was water hyacinth compost at a 25 t/ha dose. The results of this experiment showed that the treatment of ¾ compost + ¾ doses of N, P, K, and S was the best in increasing available S (26.79 mg kg^{-1}), S uptake (7.03 mg/plant), protein content (0.95%), colors and shallot yield (number of tubers, fresh weight, and dry weight) on Inceptisols from Jatinangor.

Keywords: Compost · N, P, K, S Fertilizer · Shallots · Water hyacinth

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Introduction

Indonesia has agricultural land dominated by the Inceptisol soil order, with an area that reaches 37,5% of Indonesia's land area or around 70,52 million ha (Setyastika and Suntari, 2019). West Java has an area of land with the Inceptisol order of 2.12 million ha that is used for agricultural activities (Puslittanak, 2000). Inceptisol soil has relatively low fertility and chemical properties, among other problems of acidic pH, high clay content, and the surface layer being easily washed away (Sudirja et al., 2006). The nutrient content of Inceptisol soil from Jatinangor in N-total 0.10% is included in the low category; P_2O_5 58.28 mg 100 kg^{-1} classified as high; K_2O 31.54 mg 100g^{-1} moderate; and SO_4 41.42 mg kg^{-1} which is low. Soil fertility plays an essential role in increasing crop productivity. Soil fertility can be increased by inputting nutrients into the soil. One of the treatments to increase soil fertility is optimal fertilization. Fertilizers commonly used are organic and inorganic fertilizers. The provision of organic and inorganic fertilizers is better than only providing inorganic fertilizers.

Research by Ramadhan et al. (2018) suggests that applying organic and inorganic fertilizers can increase the dry weight of shallots by 40.79% or 16.89 t/ha compared to without using organic fertilizers. Excessive application of inorganic fertilizers will cause damage to the physical, chemical, and biological properties of the soil. It will inhibit the activity of beneficial microorganisms in the soil. Hence, efforts need to be made to make additional inputs of organic fertilizers to reduce synthetic fertilizers' use on soil and plants.

The use of organic fertilizers will benefit the soil because they can improve the soil's physical, chemical, and biological properties and increase the activity of soil microorganisms and support environmental sustainability. One of the organic fertilizers that can be used is compost. Compost is an organic material that can be used to improve soil properties. Compost is obtained from plant residues and animal waste that microorganisms have decomposed to contain essential nutrients for plants (Setyorini et al., 2006) and relatively stable and straightforward genetic material (Sahwan, 2016).

Water hyacinth {*Eichhornia crassipes* (Martt.) *Solm*} is one source of organic matter that can be used to make compost. Water hyacinth is an aquatic weed that can be a source of problems for the environment, especially in water areas. Water hyacinth is a hyperaccumulator plant that can absorb heavy metals both in soil and in water (Widyasari, 2021), so it is necessary to do an initial analysis of heavy metal content in water hyacinth plants if it is to be used as a source of compost. Water hyacinth compost contains 16.94% C-organic, 13.56 C/N, 1.25% N, P_2O_5 1.31%, K_2O 0.39%, and S 6 122.68 mg kg^{-1}. Water hyacinth compost has a higher sulfur (S) content than compost made from various other raw materials, so it becomes an advantage of water hyacinth compost (Sofyan, 2014a). The high nutrient content in water hyacinth compost will support and improve soil quality and plant productivity, including shallots.

Shallot is a horticultural commodity with various benefits that can be used as spices, seasonings, and food additives. In addition, shallots are enriched with substances that are beneficial to humans, such as minerals, multivitamins, and sulfur which can prevent cancer (Syamsuddin and Hasrida, 2019). Thus, shallots are in demand and a mainstay of Indonesian society. Data from the Ministry of Agriculture (2020) showed that the consumption of red onions in Indonesia in 2019 reached 2.72 kg/capita/year.

Shallot production data for 2019 states that the demand for shallots will continue to increase in line with the community's increasing needs due to the population growth rate. Indonesia's shallot productivity potential can reach 20 t/ha. Data from the Central Bureau of Statistics (2019) states that the productivity of Indonesian shallots in 2019 only reached 9.93 t/ha, although the production increased in 2020. Therefore, efforts are needed to increase the productivity of quality shallot crops to maximize the potential for shallot production.

The quality of shallots is characterized by their characteristic red color, dense tubers with an oval shape, spicy taste, and distinctive aroma (Sumarni and Hidayat, 2014). The use of high-quality and high-yielding seeds is one of the factors in efforts to increase the productivity of quality shallots. Wide shallot varieties have been released with high yield potential and adaptability and can be planted in the lowlands, namely the Batu Ijo variety (Rahman et al., 2016). In addition to varieties, soil conditions and fertilization also affect the yields of shallot plants (Afliana, 2017). Shallots will grow well if planted

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in loose soil with high humus, well aerated, and quickly provides water. The desired soil type is clay with a balanced fraction of clay, sand, and silt, has an acidity (pH) of 5.6 – 6.5, and is most suitable for planting on alluvial soil (Hanafiah, 2008).

Sulfur is essential nutrient plants need (Zhao et al., 2015). Shallots require sulfur elements for growth and development (Assefa et al., 2015). Giving sulfur increases the fresh weight of the resulting shallot bulbs (McCallum *et al*., 2005). Lack of S will result in plant stunting, thinness, and leaves that will turn yellow.

The amino acids cystine, cysteine, and methionine contain sulfur which is essential in the formation, function, and structure of proteins. As a constituent of protein, Amino acids have a role for plants in transporting other substances, regulating organismal activity, cell response to stimuli, movement and protection against disease, and accelerating chemical reactions selectively (Neil, 2004). One source of S is water hyacinth compost. Water hyacinth has a reasonably high protein content, between 12-18%, with a relatively complete amino acid content (Little, 1997).

This study aimed to determine the effect and obtain the doses of water hyacinth compost and N, P, K, S fertilizers, which could increase available S, S uptake, protein content, and yield of shallots on Inceptisol Jatinangor soil. In order to reduce the use of inorganic fertilizers, a dose of ¾ compost + ¾ compost is the best treatment for increasing shallot crop yields.

Materials and Methods

This experiment was carried out on plastic-covered land in the Experimental Field of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency. The analysis of water hyacinth, soil, and plant compost was carried out at the Soil Fertility and Plant Nutrition Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java. This experiment was carried out from June to October 2021.

The tools used in this experiment are polybag size 30 cm x 30 cm, analytical balance, caliper, cutter, laboratory equipment such as a spectrophotometer, digestion blocks, and other laboratory equipment.

The materials used in this experiment were soil media of the order Inceptisol originating from Jatinangor, water hyacinth compost, fertilizer Urea (46% Nitrogen), SP-36 (36% Phosphate), KCl (60% Potassium), dan ZA (21% Nitrogen, 24% Sulfur)with various doses that are adjusted to the treatment, the Batu Ijo variety of shallot seeds, and various chemicals needed such as HNO_3, $HClO_4$, $BaCl_2$-tween, activated carbon, sodium acetate, as well as other materials needed.

Experimental Design. This experiment was carried out using a Randomized Block Design (RBD), which consisted of one control treatment, two treatments giving recommended doses of fertilizers by Petrokimia (2011) for shallots (N, P, K, and ZA fertilizers as much as 200 kg Urea, 500 kg ZA, 300 kg SP-36, and 200 kg KCl), four combination treatments between water hyacinth compost and fertilizer N, P, K, S. Each treatment was repeated four times with two experimental units so that a total of 56 polybags with a spacing of 20 cm x 20 cm. The following is the treatment given:

Table 1. Arrangement of water hyacinth compost and N, P, K, S fertilizer treatment for shallots

Treatment	Information
A	Control
B	¾ N, P, K, S recommendations
C	1 compost
D	¼ compost + ¾ doses of N, P, K, S
E	½ compost + ¾ dose N, P, K, S
F	¾ compost + ¾ doses of N, P, K, S
G	1 compost + ¾ dose N, P, K, S

Analysis Plan. The experimental data were statistically processed using Fisher's test at a 5% significance level using the SPSS version 22.0 application. If the effect is significant, the test is continued with Duncan's Multiple Range Test or *Duncan Multiple Range Test* at an actual level of 5%.

Compost Making. The compost used comes from 70 kg of water hyacinth. Production begins with chopping or cutting the water hyacinth to a size of 3-5 cm to speed up the decomposition process. Then, 350 g of Orgadec bio activator was given, which was stirred evenly. The composting process was carried out anaerobically and observed for six weeks.

Preparation of Planting Media and Planting. The experiment was initiated by carrying out a complete analysis of the initial soil to obtain information on the soil's actual physical and chemical properties. The soil sample in this experiment was the soil of the order Inceptisols from Jatinangor, Sumedang Regency, West Java. Composite soil was taken at a 0-20 cm depth of 500 g. Drying was carried out on the planting medium, then pounded and filtered using a 2 mm sieve to obtain uniform soil grains. After that, the weight of the soil was carried out as much as 8 kg for each polybag so that the amount of land used in this experiment was 448 kg. Soil with the provision of compost fertilizer treatment is homogenized beforehand. The compost used in this experiment was water hyacinth compost at a dose of 25 t/ha or the same with 125 g/polybag so that the required water hyacinth compost is 2 kg.

Planting and Fertilization. Shallot planting is done by making a planting hole punched in the ground and planting one bulb per polybag. The size used is 30x30 cm. To stimulate tuber growth, cut the tip of the tuber by ¼ part of the tuber. Before planting, the tubers are cleaned first of the outer skin and remaining roots.

Fertilization is carried out using inorganic fertilizers, namely N, P, K, and ZA fertilizers which are applied at the beginning of planting and several intervals. At the time of planting, SP-36 and KCl fertilizers were applied. The frequency of Urea and ZA fertilization was twice, which was applied seven days after planting and 21 days after planting. Fertilizer application is made by: a sideband or next to a plant. The doses given are based on recommendations Petrokimia (2011) for shallots, namely N, P, K, and ZA fertilizers, as much as 200 kg of Urea, 500 kg of ZA, 300 kg of SP-36, and 200 kg of KCL. Each treatment was given a different dose.

Maintenance. Plant maintenance in this experiment was carried out with activities that included watering, weeding, controlling plant pests, and replanting. Watering is done in moderation according to field capacity and is done in the morning. Weeding is done physically by directly pulling the weeds around the planting medium. Control of plant-disturbing organisms is carried out by removing directly (manually) and applying (chemical) pesticides. Replacement of seeds that die at 0-14 days after planting and stitching is done.

Sampling. Shallots that have experienced the maximum vegetative phase or shallots aged 42 days after planting are then sampled to analyze the soil's chemical properties and nutrient absorption. Each treatment in all repetitions is observed. Samples are obtained by taking the culture media on polybags and homogenizing them to analyze soil chemical properties. Part of the plant is taken as a sample to analyze the nutrients in the plant tissue. The tuber yield was measured when the shallots were harvested late by weighing the tubers of each treatment.

Harvesting. Shallots have characteristics when they are ready for harvest, namely the base of the stem is soft and dry, the leaves fall ± 80% and turn yellow, the bulbs are fully filled, sticking out to the ground, and the color is purplish-dark red when it reaches 67 days after planting. The harvesting process is carried out when the soil conditions are dry, and the weather is sunny. After harvesting, the shallots are cleaned by separating them from the remaining soil attached and tying them to the surface of the leaves to make it easier to handle the harvest. Shallots that have been harvested are dried. The drying process was carried out for 14 days at room temperature. After air drying, weighing is carried out to determine the dry weight of the shallots.

Results and Discussion

S-available. The content of S-Available in Inceptisol soil from Jatinangor can be seen in Table (2). Based on the analysis of the available S content in shallots, it was shown that fertilizing with N, P, K, and S fertilizers with a combination of N, P, K, and S fertilizers and water hyacinth compost had a significant effect on available S.

This experiment produced the highest available S in the treatment of 1 compost + ¾ doses of N, P, K, S of 29.19 mg.kg^{1} but not significantly different from the treatment of ¾ compost + ¾ doses of N, P, K, S. Giving 1 compost + ¾ doses of N, P, K, S is the best treatment because there is a cooperation between inorganic fertilizers and organic fertilizers (compost) in providing the nutrients needed by shallot plants. The lowest available S was in the control treatment of 5.69 mg.kg^{-1}.

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Table 2. Effect of water hyacinth compost and N, P, K, S Fertilizer on S-availability of Inceptisol soil from Jatinangor

Treatment	S-available (mg kg^{-1})
A (Control)	5.69 a
B (¾ N, P, K, S recommendations)	13.72 b
C (1 compost)	11.22 b
D (¼ compost + ¾ dose N, P, K, S)	17.34 c
E (½ compost + ¾ dose N, P, K, S)	25.49 d
F (¾ compost + ¾ dose N, P, K, S)	26.79 de
G (1 compost + ¾ dose N, P, K, S)	29.19 e

Note: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

The application of water hyacinth compost affects increasing the availability of S obtained from the decomposition of water hyacinth plants by microorganisms so that there is a balance of nutrients in the soil. The results of laboratory analysis showed that the S content in the water hyacinth compost used in this experiment was 6122.68 $mg.kg^{-1}$.

The increase in available S-content in the soil is due to the role of beneficial microorganisms such as *Thiobacillus* sp., which is an S-oxidizing bacterium, thereby stimulating the availability of S in the soil. Bacteria *Thiobacillus* sp. has an essential role in the soil as it oxidizes unavailable S to become available to plants through of stage $S^{O} \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-}$ (Yang et al., 2010). Soil that was incubated with S-oxidizing bacteria gave result SO_4^{2-} which was higher in a faster time than soil that was not incubated with S.

In addition to compost, N, P, K, and S fertilizers can also provide S in the soil directly. The application of S fertilizer can increase the availability of S, which is higher than without S fertilizer (Matamwa et al., 2018). This is in accordance with research conducted by Nurhidayati et al. (2013), which stated that the availability of S by giving ZA 900 $mg.kg^{-1}$ capable of increasing S-available 30.50 $mg.kg^{-1}$ higher by 76% than without giving ZA, which is equal to 3.98 $mg.kg^{-1}$. Direct fertilizer application can provide direct availability of nutrients absorbed in the soil so that S availability increases.

S Uptake. Based on the S uptake analysis presented in Table 3, S uptake shows that the application of N, P, K, and S fertilizers and a combination of N, P, K, S fertilizers and water hyacinth compost have a significant effect on S uptake. Table 3 shows that the treatment 1 compost + ¾ doses of N, P, K, S resulted in the highest S uptake of 7.62 mg/plant while the control treatment produced the lowest S uptake of 4.48 mg/plant.

There is a relationship between the high S absorption resulting from the treatment and the availability of S in the soil (Hardjowigeno, 2010). This relationship can be proven in Table 3 that the treatment of 1 compost + ¾ dose of N has the highest available S compared to the other treatments in line with the results of S absorption in Table 3, which shows that the treatment of 1 compost + ¾ dose of N has the highest S uptake. The results of the S uptake of 1 compost + ¾ doses of N, P, K, S were not significantly different from the treatment of ¾ compost + ¾ doses of N, P, K, S. Based on the S uptake data (Table 3), the ¾ compost + ¾ doses N, P, K, S was the best treatment because it was able to increase S uptake by reducing the use of fertilizers.

Table 3. Effect of Water Hyacinth Compost and N, P, K, S Fertilizers on S Uptake of Inceptisol Soils from Jatinangor.

Treatment	S uptake (mg/plant)
A (Control)	4.48 a
B (¾ N, P, K, S recommendations)	5.72 bc
C (1 compost)	5.42 b
D (¼ compost + ¾ dose N, P, K, S)	5.84 bc
E (½ compost + ¾ dose N, P, K, S)	6.52 cd
F (¾ compost + ¾ dose N, P, K, S)	7.03 de
G (1 compost + ¾ dose N, P, K, S)	7.61 e

Note: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

Applying water hyacinth compost and N, P, K, and S fertilizers gave high S absorption results when compared to the control treatment because it could supply nutrients, including S, in the soil so that more S in the soil could be absorbed. Pradhan et al. (2015) also showed that N, P, K, S (150, 50, 80, 45 kg/ha) gave high S uptake of up to 9.8 mg/plant four times greater than N, P, K fertilizer treatment recommendations (150, 50, 80 kg/ha). According

the research of Sofyan (2014b) also applied 166.7 kg/ha of ZA fertilizer and 20 t/ha of water hyacinth compost, demonstrating the effectiveness of giving water hyacinth bokashi to lowland rice plants which were able to increase S uptake three times greater than the control treatment.

Plants need S because it is an essential nutrient to optimize plant growth. Sulfur has a role in the synthesis of proteins, vitamins and is closely related to the N metabolism needed by plants. A deficiency of element S will result in the growth of shallot plants not being optimal because shallot uptake is not optimal, causing a decrease in the yield and quality of shallots (Pradhan et al., 2015). In addition, the addition of water hyacinth compost affects improving soil physical properties such as soil structure, aeration, and better infiltration. Shallot plants require good soil conditions to grow optimally (Hendrawan et al., 2018). Good soil properties can support root growth to expand the range of nutrient absorption areas in the soil so that S uptake increases. Infiltration factors play an essential role in increasing S uptake by plants through mass flow.

Protein Content. The application of N, P, K, and S fertilizers and the combination of N, P, K, and S fertilizers with water hyacinth compost significantly affected shallot protein content. Table 4 presents the results of the statistical test of shallot protein content, showed that the treatment of 1 compost + ¾ doses of N, P, K, S resulted in protein levels tending to be higher than the other treatments of 3.07% but not significantly different from the treatment of ¾ compost + ¾ doses of N, P, K, S of 2.95 %. The control treatment had a lower protein content of 1.87% but was not significantly different from the 1 compost treatment of 1.97%.

The high protein content is affected by the availability of S in the soil, which plants absorb. Provision of compost and N, P, K, and S fertilizers have a role in increasing the protein content of shallots, especially elements N and S. The ZA fertilizer given to the treatment contains 24% S compounds and 21% N in the form of ammonium (Kiswondo, 2011). Nitrogen is a constituent element of protein (Nugraha, 2010) and plays a role in plant growth. Sulfur is a constituent of amino acid compounds that form proteins that function in the formation of chlorophyll and metabolic reactions of proteins, carbohydrates, and fats (Winarso, 2005). Amino acids in plant protein contain 90% of its element S. These amino acids function in improving the quality of shallots, namely aroma.

Table 4. The effect of water hyacinth compost and N, P, K, S fertilizer on shallot total protein in Inceptisol soil from Jatinangor

Treatment	Protein Total (%)
A (Control)	1.87 a
B (¾ N, P, K, S recommendations)	2.57 b
C (1 compost)	1.97 a
D (¼ compost + ¾ dose N, P, K, S)	2.72 bc
E (½ compost + ¾ dose N, P, K, S)	2.74 bc
F (¾ compost + ¾ dose N, P, K, S)	2.95 cd
G (1 compost + ¾ dose N, P, K, S)	3.07 d

Note: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

The compost treatment + ¾ doses of N, P, K, S was the treatment that produced the highest protein content but was not significantly different from the ¾ compost treatment and ¾ doses of N, P, K, S, which was 2.95%, so the shallot crop needs in increasing protein are still sufficient by administering ¾ compost + ¾ doses of N, P, K, S. Based on these results, the best treatment that increases protein levels of shallots is the treatment of ¾ compost + ¾ doses of N, P, K, S due to the use of more fertilizer A little.

Red Onion Bulb Color. The color assessment of shallot plants through the chromameter test showed that the application of water hyacinth compost and N, P, K, and S fertilizers affected the color values of L* (brightness), b* (yellow-blue), and a* (blue-yellow). In this test, the L* values range from 0-100, a* and b* values have the same range from -100 to 100. A* values with negative values indicate green and cheerful indicate red, while b* with negative values indicating blue and positive values indicating yellow.

The color of shallots is produced from anthocyanin compounds. Anthocyanin compounds are organic chemical compounds that release color pigments such as red, blue, orange, and black in various parts of plants, namely tubers, seeds, flowers, vegetables, and fruit (Priska et al., 2018).

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Table 5. The effect of water hyacinth compost and N, P, K, S fertilizers on the color of shallot bulbs in Inceptisol soil from Jatinangor

Treatment	Color Value		
	L* (Brightness)	a* (Red Green)	b* (Blue-Yellow)
A (Control)	46.56 bc	22.65 ab	-5.22 a
B (¾ N, P, K, S recommendations)	46.20 bc	21.07 a	-5.71 a
C (1 compost)	43.43 a	23.96 ab	-4.82 a
D (¼ compost + ¾ dose N, P, K, S)	44.47 ab	24.45 b	-5.01 a
E (½ compost + ¾ dose N, P, K, S)	47.73 c	21.77 ab	-8.76 c
F (¾ compost + ¾ dose N, P, K, S)	46.14 bc	21.59 ab	-8.28 bc
G (1 compost + ¾ dose N, P, K, S)	48.12 c	21.28 ab	-6.35 ab

Note: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

Table 6. Effect of water hyacinth compost and N, P, K, S fertilizer on shallot yield in Inceptisol soil from Jatinangor

Treatment	The Number of Tubers	Fresh Weight of Yield (g)	Dry Weight of Yield (g)
A (Control)	5.0 a	92.89 a	67.46 a
B (¾ N, P, K, S recommendations)	7.0 b	107.27 b	79.42 a
C (1 compost)	7.0 b	98.09 a	75.79 a
D ($\frac{1}{4}$ compost + ¾ dose N, P, K, S)	8.0 b	115.14 c	84.37 a
E (½ compost + ¾ dose N, P, K, S)	8.0 b	121.93 cd	101.72 b
F (¾ compost + ¾ dose N, P, K, S)	8.0 bc	124.82 de	102.90 b
G (1 compost + ¾ dose N, P, K, S)	9.0 c	132.26 e	107.01 b

Note: The numbers followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% significance level.

Anthocyanins will work together with thiosulfate to stabilize the anthocyanin content in shallots. Thiosulfate compound also plays a role in the formation of color pigments in shallots (Sukasih and Mukadad, 2018). The element S forms the thiosulfinate compound (Forney, et al., 2010).

Water hyacinth compost also contains a high element of S compared to other composts, which affects the availability of thiosulfate compounds. This is also assisted by the availability of S in the soil, which results in increased S uptake in plants.

The low yield of shallots is affected by the absence of input given to the soil resulting in low nutrient availability and nutrient uptake in shallots. Nutrients that are not available will inhibit the growth and development of shallots.

Nutrient deficiency results in low shallot yields due to suboptimal growth and development of shallot plants. A lack of element N will inhibit the process of cell division and the formation of chlorophyll in plants (Lestari and Palobo, 2019). A deficiency of P and S elements

The number of bulbs, fresh weight, and dry weight measured the shallot yield parameters. These parameters have been tested statistically, which is presented in Table 6. Based on the results of the statistical test results of shallots, it can be seen that the control treatment is the treatment with the lowest results on all observation parameters with a total of 5 bulbs; a fresh weight of 92.89 g; and a dry weight of 67.46 g compared to other treatments but not significantly different from the recommended ¾ N, P, K, S treatment; 1 Compost; and ¼ compost + ¾ doses of N, P, K, S. causes plants to become stunted, thin, and have few leaves (Wati et al., 2015). A lack of K elements results in enzyme activity, protein formation, cell enlargement, and photosynthate transport to tubers (Lestari and Palobo, 2019).

Treatment of 1 compost + ¾ doses of N, P, K, S was the highest treatment with nine tubers; the fresh weight of 132.24 g; and dry weight of 107.01 g but not significantly different from the treatment with ¾ compost + ¾ doses of N, P, K, S. Thus, the ¾ compost + ¾ N, P, K, S treatment

was the best treatment because it increased yields and reduced fertilizer use. Organic and inorganic fertilizers work together to provide the nutrients needed by plants. The addition of nutrients needed by plants can help synthesize proteins, nucleic acids, chlorophyll, and photosynthesis in plants (Havlin et al., 2016).

There was an increase in shallots because the required nutrients were met, such as N, P, K, S, and water hyacinth compost from the treatment given. Application of fertilizers N, P, K, S, and water hyacinth compost produced higher plant height, number of tubers, fresh weight, and dry weight of tubers and was significantly different compared to no treatment and compost application alone.

Each nutrient provides its role to support the growth and development of shallot plants so that it affects shallot yields. Nutrients N which support the vegetative period and protein formation in shallots, P elements which support root development, and K, which has a role in root and stem growth and protein formation (Havlin et al., 2016). The S element is essential in enlarging tubers and the number of tubers produced (Herwanda et al., 2017).

Water hyacinth compost also plays an essential role in increasing shallot yields. The application of compost can improve the soil's physical properties so that it affects the growth and yield of shallots on Inceptisol soil. Compost plays a role in soil aeration so that the soil pores increase, which increases air availability in the soil so that root penetration in absorbing nutrients increases. This also impacts the process of root respiration, which influences the growth and development of plant root systems (Hardjowigeno, 2010).

Conclusion

Applying water hyacinth compost combined with N, P, K, S fertilizers significantly increased the uptake of S, S-available, Protein, and yield of shallots, including color and fresh weight, dry weight, and the number of tubers.

Treatment of ¾ compost (18.75 t/ha) + ¾ doses of N, P, K, S (150 kg: 225 kg: 150 kg: 375 kg) was the best treatment capable of increasing available S (26.79 mg kg^{-1}), S uptake (7.03 mg/plant), protein content (2.95%), and shallot yield (fresh weight, dry weight, and the number of tubers) and red-purple color.

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Genetic variability, heritability, and path analysis for agronomic characters in hybrid maize

Abstract. Genetic parameters, which include genetic variability, heritability, and correlation between characters, are essential factors in the selection process. This study aims to: 1) determine the genetic variability and heritability for agronomic characters in hybrid maize and 2) determine the characteristics that directly affect hybrid maize yield. The experiment was conducted at Indonesian Cereal Research Institute (ICERI) Maros from June to October 2021. Fourteen maize hybrids genotypes were arranged in a randomized block design (RBD) with four replications. The observed traits were plant height, ear height, stalk diameter, leaf angle, leaf length, leaf width, days to anthesis, days to silking, days to maturity, number of harvested plants, number of harvested ears, fresh ear weight, shelling percentage, moisture content, ear length, ear diameter, number of rows per ear, number of seeds per row, 1000 seeds weight and yield. The results showed that agronomic characters with high heritability and broad genetic variability were plant height, ear height, leaf angle, leaf length, ear diameter, and 1000 seeds weight. The characters that most influence final yield were fresh ear weight and shelling percentage

Keywords: Genetic variability · Heritability · Maize · Path analysis

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Introduction

Selection is the crucial point in a plant breeding program. Selection effectivity can be increased by noticing genetic and phenotypic variability, heritability, and the correlation between traits in every stage (Nzuve et al., 2014). Variability is the primary capital for plant breeders in improving plant characteristics. The variability in a population was due to genetics, environment, and interaction between genetic and environmental factors. The variability used in plant breeding is variability due to genetic factors (Azrai, 2013). A genetic variability is number that measures the appearance variation due to genetic factors. Genetic variability can describe individual variation in a population(Barmawi et al., 2013; Kristamtini et al., 2014). The higher trait genetic variability gives the greater desired good traits combination chance so the plant breeding program success will be increased (Hapsari, 2016).

One of the things to consider in the selection is heritability. Heritability is the proportion of genetic variance to a trait's total variance, expressed in phenotypic performance, and can be inherited by subsequent generations (Qosim, 2018). Heritability values ranged from 0-1. Higher heritability values indicate more significant genetic and small environmental influences on traits and vice versa. Selection can be made in the early generations for traits with high heritability values, while traits with low heritability values are selected for advanced generations (Rini et al., 2018). A selection guided by heritability can increase the selection program's success in obtaining better results.

There are two ways to select a character. Selection can be made by direct selection and indirect selection through characters that correlate with the desired character. The correlation value will make indirect selection easier. The correlation coefficient provides a relationship between characters and valuable information about the level and direction of selection (Bechere et al., 2014; Maftuchah et al., 2015). The correlation coefficient between traits has an essential meaning in selection. Selection will be effective if there is a close relationship between the estimating character and the desired character.

Yield selection can be based on the correlation between yield and agronomic character. Occasionally, there is bias in the selection because the agronomic character was correlated with each other, so it is necessary to spell the correlation out into the direct and indirect effects. Path analysis can solve that problem by spelling out the character correlated to yield (Manggoel et al., 2012). Path analysis besides being used for food crops including soybeans (Saputra et al., 2016), rice (Kartina et al., 2016), maize (Kmail et al., 2017)) is also commonly used for pineapple (Donazzolo et al., 2017), chilli (Sa'diyah et al., 2020) even on plantations such as cocoa (Sari and Susilo, 2013), sugarcane (Baffa et al., 2014) and jatropha (Hartati et al., 2012).

Studies regarding genetic parameters commonly carried out in crop plant breeders include mung bean (Hapsari, 2016), soybean (Karyawati et al., 2019), rice (Kristamtini et al., 2014) and maize (Mhoswa et al., 2016). Even though maize heritability for character agronomic (Maphumulo et al., 2015) dan shelling percentage (Adriani et al., 2015) have been researched; however, it is done separately so that information on variability, heritability, and correlation still needs. This is the reason behind the need to study hybrid maize genetic parameters. This research was conducted to determine the genetic variability, heritability, and characters that directly affect hybrid maize yield. The information obtained can help a selection program designed for hybrid maize breeding.

Method

This experiment was conducted at Indonesian Cereal Research Institute (ICERI) Maros from June to October 2021. Fourteen maize hybrids genotypes (Table 1) were arranged in a randomized block design (RBD) with four replications.

The experimental dimension plot was 1.4 m x 5 m, with a spacing of 50 cm x 20 cm, with one plant per hole, so there were 25 plants per row. The first fertilization was done seven days after planting (DAP) with 200 kg of urea and 300 kg of Phonska per ha. Second fertilization at 30 DAP with 200 kg of urea per ha. Plant maintenance includes weeding, irrigation, and control pest management. Harvesting was done in the middle two rows of the experimental plot.

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Table 1. Hybrid maize in present study

No	Hybrid	Crossing
1	NGN 1	P4 X P8
2	NGN 2	P8 X P7
3	NGN 3	P8 X P2
4	NGN 4	P7 X P2
5	NGN 5	P10 X P2
6	NGN 6	P8 X P6
7	NGN 7	P7 X P5
8	NGN 8	P7 X P3
9	NGN 9	P10 X P8
10	NGN 10	P4 X P2
11	NGN 11	MAL 03 X P7
12	JHANA 1	MAL 03 x CY 15
13	BISI 18	FS46 x FS17
14	NK 7328	NP5150 x NP5139

The observed character are plant height (PH), ear height (EH), stalk diameter (SD), leaf angle (LA), leaf length (LL), leaf width (LW), days to anthesis (DA), days to silking (DS), days to maturity (DM), number of harvested plants (NHP), number of harvested ears (NHE), fresh ear weight (FEW), shelling percentage (SP), moisture content (MC), ear length (EL), ear diameter (ED), number of rows per ear (NRE), number of seeds per row (NSR), 1000 seeds weight (1000 SW) and yield (Y) which was corrected to 15% moisture that converted to units per hectare using the formula (Sujiprihati et al., 2006):

$$\text{Yield (t /ha)} = \frac{10.000}{\text{HA}} \text{ X} \frac{\text{100-GM}}{85} \text{ X FEW x SP} \div 1.000$$

HA = Harvested area (m^2)
GM = Grain moisture (%)
EHW = fresh ear weight (kg)
SP = Shelling percentage (%)

Observed data were analyzed based on Gomez and Gomez (1983) method. Genotypic and phenotypic variability was computed from an analysis of variance (Hallauer *et al*. 2010), presented in Table 2.

Table 2. Analysis of variance with Expected Mean Square

Source of variation	Degree of freedom (df)	Mean Square (MS)	Expected Mean Square (EMS)
Replication	r-1	MSr	$\sigma^2\varepsilon + g\sigma^2r$
Genotype	g-1	MSg	$\sigma^2\varepsilon + r\sigma^2g$
Error	(g-1)(r-1)	MSε	$\sigma^2\varepsilon$

According to Table 2, then genotypic and phenotypic variance can be computed as follows: $\sigma^2 g = \frac{MS_g - MS_\varepsilon}{r}$, $\sigma^2 p = MS_g + \varepsilon$

Broad-sense heritability (H^2) was estimated according to the procedure suggested by Allard (1960):

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p}$$

H^2 value was grouped according to Stansfield (1983) as follows:
$H^2 > 0{,}5$ high, $0{,}2 \leq H^2 \leq 0{,}5$ medium, $H^2 < 0{,}2$ low.
Standard deviation of genotypic variance:

$$\sigma_{\sigma_{\bar{g}}^2} = \sqrt{\frac{2}{r^2}\left(\frac{{MS_g}^2}{\text{Genotype's df+2}} + \frac{{MS_\varepsilon}^2}{\text{Error's df+2}}\right)}$$

Genetic variability was categorized as low when $\sigma^2_g < 2\sigma_{\sigma}{}^2_g$ and high when $\sigma^2_g \geq 2\sigma_{\sigma}{}^2_g$ (Pinaria et al., 1995).

Pearson correlation was used to define the correlation between characters using the formula:

$$r_{xy} = \frac{Cov_{xy}}{\sqrt{Var_x . Var_y}}$$

Where:
r_{xy} = Correlation between x character and y character,
Cov_{xy} = Covariance between x character and y character,
Var_x = Variance of x character
Var_y = Variance of y character

The direct and indirect effect of a character agronomic to yield was determined by conducted path analysis. Path analysis was only carried out on characters that influence yield. The characters that influence yield are decided by stepwise regression analysis. Path analysis was conducted according to Singh and Chaudhary (1979):

$$\begin{bmatrix} r_{11} & r_{21} & \cdots & r_{1p} \\ r_{12} & r_{22} & \cdots & r_{2p} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ r_{1p} & r_{2p} & \cdots & r_{pp} \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \\ \cdots \\ \cdots \\ C_p \end{bmatrix} = \begin{bmatrix} r_{1y} \\ r_{2y} \\ \cdots \\ \cdots \\ r_{py} \end{bmatrix}$$

R_x C_i R_y

Ci (direct effects) were calculated by the formula:
$C_i = R_x^{-1} . Ry$

Effects that a model cannot define were classified as the residual effect calculated by the formula:

$C_s = \sqrt{C_s^2}$; $C_s^2 = C_i' R_x$

Whereas:

R_x = Correlation matrix of the dependent variable

R_x^{-1} = Inverse matrix of Rx.

C_i = Path coefficient vector that shows the direct effect of each independent variable on the dependent variable

R_y = Correlation coefficient vector between independent variable X_i (i=1, 2…, p) and dependent variable Y

C_s = residual effect or error

C_i' = Transpose matrix of C_i

Data analysis was performed using Statistical Tool for Agricultural Research (STAR) Version: 2.0.1 dan Microsoft Excel software.

Result and Discussion

Analysis of variance for observer variable. The results showed genotype character variability except for stalk diameter, days to silking, days to maturity, number of harvested plants, number of harvested ears, moisture content, and number of seeds per row (Table 3). Characters that do not show significant differences besides having low variance also show a narrow range of values. The difference shown in the analysis of variance is a phenotypic variability. Furthermore, it is necessary to carry out further studies to find out how much the genetic factors influence each character's variability (Nur *et al*. 2013).

The coefficient of variability (CV) in this research is between 1.4% (days to maturity) to 24.9% (yield). CV value represents the variability population in a study. CV shows precision values of research. Karuniawan et al., (2017) mention that higher CV values mean lower precision study and vice versa. Almost all characters show CV values below 20% except fresh ear weight and yield. This means that this study has high precision. CV values between 20-25% in agriculture study is reasonable. Prayudha et al. (2019) say that factors affecting CV values are experimental design, plant characteristics, observed variables, and the experimental environment.

Table 3. Analysis of variance and values range of observed variable

Character	Mean Square (MS)				CV (%)	Values range
	Replication	Genotype		Error		
Plant height	2199.82	866.57	**	83.84	4.30	184.90-237.75
Ear height	1336.29	480.84	**	91.04	8.30	98.13-133.44
Stalk diameter	8.44	2.24		1.52	6.30	18.30-21.05
Leaf angle	0.33	42.85	**	2.90	6.80	20.35-33.30
Leaf length	54.45	121.65	**	30.44	6.20	80.1-98.95
Leaf width	1.61	1.17	*	0.57	9.30	7.13-9.32
Days to anthesis	0.35	2.94	*	1.15	2.00	52.25-55.50
Days to silking	1.78	3.24		1.86	2.50	53.75-57
Days to maturity	3.19	2.55		2.38	1.40	107.50-110.25
Number of harvested plants	5.45	4.16		3.37	5.80	30.00-33.5
Number of harvested ears	5.29	3.49		2.91	5.40	30.25-33.25
Fresh ear weight	8.53	3.35	*	1.46	22.30	3.91-6.76
Shelling percentage	23.72	92.61	*	39.09	8.00	0.65-0.86
Moisture content	23.21	30.39		17.10	14.90	22.00-31.9
Ear length	3.11	5.13	*	2.30	8.80	14.62-18.83
Ear diameter	1.94	45.51	**	11.21	8.10	31.80-45.71
Number of rows per ear	2.66	1.33	**	0.36	4.20	13.55-15.8
Number of seeds per row	10.90	12.90		8.39	8.60	29.15-35.95
1000 seeds weight	1069.18	3743.91	**	834.14	11.70	201.3-297.72
Yield	7.75	4.24	*	1.65	24.90	3.45-6.67

**= significant at P<0.01, *= significant at P<0.05, CV= coefficient of variability

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Heritability and genetic variability. The estimated heritability explained in this study is broad sense heritability. The broad sense heritability only describes genetic factors. Table 4 reveals that the observed character heritability values are varied. Low estimated heritability value is shown by days to maturity, number of harvested plants, and number of harvested ears. Low estimated heritability values indicate that these characters have low genetic potential and high environmental influences. Mathew et al. (2018) stated that minor genes usually influence characters with low heritability values The character with low heritability values is selected at advanced generations using pedigree, single seed descent, and progeny test method (Effendy et al., 2018).

Moderate estimated heritability values were displayed by stalk diameter, days to silking, moisture content and number of seeds per row (Table 4). The moderate estimated heritability values mean that additive gen influenced the character. According to Sesay et al (2016) phenotypic selection will be more effective for selection characters with moderate heritability.

Character plant height, ear height, leaf angle, leaf length, leaf width, days to anthesis, fresh ear weight, shelling percentage, ear length, ear diameter, number of rows per ear, 1000 seeds weight, and yield (Table 4) Taneva et al. (2019) mention that high estimated heritability values indicated a low environmental effect on observed character. Mass selection, backcross, and pedigree with the recurrent selection at early generation are the most suitable selection method for the character with high estimated heritability values (Yudilastari et al., 2018).

Table 4 shows that most characters observed have low genetic variability except for plant height, ear height, leaf angle, leaf length, ear diameter, and 1000 seeds weight. It means the variability among individuals in the population is low. This population's low genetic variability due to a narrow genetic background is due to the small number of origin parents. Fourteen hybrids only arranged from seven female parental lines viz P4, P7, P8, P10, MAL 03, FS46 and NP5150 and nine male parentals viz. P2, P3, P5, P6, P7, P8 CY 15, FS17 and NP5139. On another side, there were two lines (P7 and P8) as female and male parents in these hybrids. Line P7 is as female parents for three hybrids (NGN 4; NGN 7, NGN 8) and two hybrids (NGN 2 and NGN 11). Line P8 is the female parent for three hybrids (NGN 2; NGN 3, and NGN 6) and the male parent for two hybrids (NGN 1 and NGN 9). Lines P4, P10, and MAL 03 are female parents for two hybrids. Meanwhile, line P2 become the male parent for four hybrids (Table 1).

Table 4. Genotypic variance and phenotypic variance, heritability and standard deviation of genotypic variance of observed character

Character	$\sigma^2 g$	$\sigma^2 \varepsilon$	$\sigma^2 p$	H^2	$\sigma_{\sigma_g^2}$
Plant height	195.68	83.84	216.64	0.90 (High)	79.25 (High)
Ear height	97.45	91.04	120.21	0.81 (High)	44.20 (High)
Stalk diameter	0.18	1.52	0.56	0.32 (Moderate)	0.22 (Low)
Leaf angle	9.99	2.90	10.71	0.93 (High)	3.92 (High)
Leaf length	22.80	30.44	30.41	0.75 (High)	11.24 (High)
Leaf width	0.15	0.57	0.29	0.51 (High)	0.11 (Low)
Days to anthesis	0.45	1.15	0.74	0.61 (High)	0.28 (Low)
Days to silking	0.35	1.86	0.81	0.43 (Moderate)	0.31 (Low)
Days to maturity	0.04	2.38	0.64	0.07 (Low)	0.27 (Low)
Number of harvested plants	0.20	3.37	1.04	0.19 (Low)	0.43 (Low)
Number of harvested ears	0.14	2.91	0.87	0.16 (Low)	0.36 (Low)
Fresh ear weight	0.47	1.46	0.84	0.56 (High)	0.32 (Low)
Shelling percentage	13.38	39.09	23.15	0.58 (High)	8.74 (Low)
Moisture content	3.32	17.10	7.60	0.44 (Moderate)	2.94 (Low)
Ear length	0.71	2.30	1.28	0.55 (High)	0.49 (Low)
Ear diameter	8.58	11.21	11.38	0.75 (High)	4.20 (High)
Number of rows per ear	0.24	0.36	0.33	0.73 (High)	0.12 (Low)
Number of seeds per row	1.13	8.39	3.22	0.35 (Moderate)	1.27 (Low)
1000 seeds weight	727.44	834.14	935.98	0.78 (High)	345.02 (High)
Yield	0.65	1.65	1.06	0.61 (High)	0.40 (Low)

Rosminah *et al.*, (2019) mentioned that heritability is not always linear to genetic variability. This study shows that fourteen characters have high heritability and low genetic variability. Six characters have a high heritability and genetic variability. Furthermore, all the character with low and moderate heritability has low genetic variability.

A character with high heritability but a low genetic variability shows that character in the population is homogeneous. Genetic factors strongly influence performance. Characters with high heritability and genetic variability indicate that the character has a high diversity. Genetic factors have a significant influence on character. Characters with high genetic variabilty and low heritability values are marked by characters that show great variability, but the environment influences the character variability. A character with a homogenous performance is more influenced by environmental factors, which indicates a character with low heritability and variability. Selection of characters with high heritability and variability will be more efficient and effective because it will inherit great genetic advances in the future (Effendy et al., 2018; Karyawati et al., 2019). Whereas for characters with low variability, it is necessary to do a genetic induction to make selection more effective. Variabilty induction can be conducted through introduction, hybridization, and mutation (Ahsan et al., 2015).

Coefficient correlation and path analysis. Correlation is only carried out on characters that are directly related to yield. Determination of characters that are directly related to yield is carried out by stepwise regression analysis. Stepwise regression is a method that can help the analysis process get a model that highly contributes to the dependent variable (Andayani et al., 2016; Wohon et al., 2017). The stepwise regression analysis result is shown in Table 5.

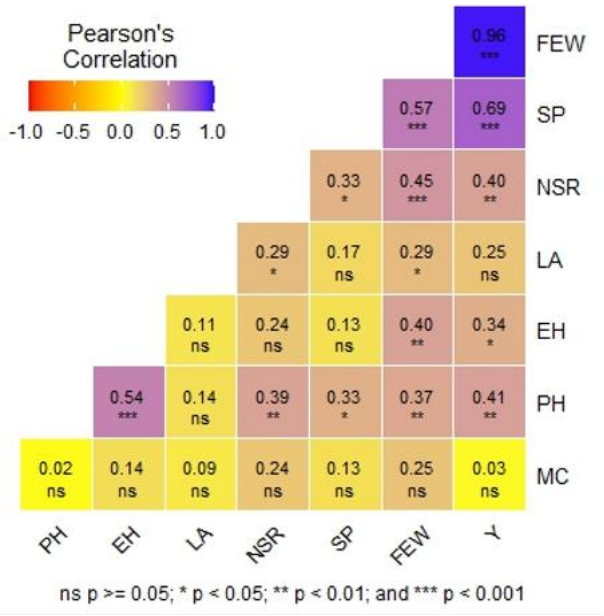
Agronomic characters with a linear relationship to yield are plant height, ear height, leaf angle, fresh ear weight, shelling percentage, moisture content and number of seeds per row (Table 5). The model that can be arranged according to that character is as follows: Y =-1.09-0.01 leaf angle+0.97 fresh ear weight+4.59 shelling percentage-0.08 moisture content-0.02 number of seeds per row. The determination coefficient value (R^2) is 0.994. This means the linear regression equation can explain 99.4% yield variation.

Table 5. Stepwise regression analysis for yield

Character	Regression	SE
PH	0	0
EH	0	0
LA	-0.01	0.01
FEW	0.97	0.02
SP	4.59	0.31
MC	-0.08	0
NSR	-0.02	0.01
intercept	-1.09	0.32

R2=0,994, PH=Plant Height, EH=Ear Height, LA=Leaf Angle, FEW=Fresh Ear Weight, SP=Shelling Percentage, MC=Moisture Content And NSR=Number of Seeds per Row

Figure 1 reveals that five characters have a significant correlation, and two characters do not have a significant correlation to yield. Plant height, ear height, fresh ear weight, shelling percentage and number of seeds per row have significant correlations. Meanwhile, leaf angle and moisture content were not significant.



PH=Plant Height, EH=Ear Height, LA=Leaf Angle, FEW=Fresh Ear Weight, SP=Shelling Percentage, MC=Moisture Content And NSR=Number of Seeds per Row

Figure 1. Pearson correlation of maize hybrid character

All of the characters with significant correlation show a positive coefficient. Positive coefficient correlation informs that if there is a change in yield component character will follow yield change in the same direction. This means

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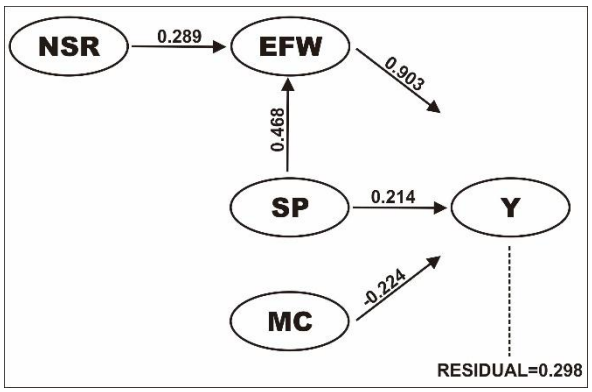
an increase in the yield component character will be followed by a yield increase and vice versa (Bewick et al., 2003). Fresh ear weight has a very strong correlation to yield (r=0.96) correlation between shelling percentage and yield is categorized as strong (r=0.69). A medium correlation was found in plant height (0.41) Silva et al., (2016) and (Raut et al., 2017) found a moderate positive correlation between plant height and yield. A weak positive correlation was found in ear height (0.34) and number of seeds per row (0.40). Idris et al. (2018) also reported a weak correlation between ear height and number of seeds per row on yield in lokal lebo maize cultivar.

The agronomic characters that correlated to yield also correlate with the other characters. that correlation makes the bias for deciding the actual yield component character effect to yield. The correlation coefficient only illustrates the close relationship between the two characters. However, it cannot explain the magnitude and direction of change because the correlation coefficient does not describe the causal relationship (Gomez and Gomez, 1983). Path analysis can solve this problem by measuring the actual effects by breaking them down into direct and indirect effects (Singh et al., 2017).



PH=Plant Height, EH=Ear Height, LA=Leaf Angle, FEW=Fresh Ear Weight, SP=Shelling Percentage, MC=Moisture Content And NSR=Number of Seeds per Row

Figure 2. Path analysis of agronomic character to yield

Characters that display a direct effect to yield are fresh ear weight, shelling percentage, and moisture content (Figure 2). Fresh ear weight and shelling percentage have a positive direct effect, while moisture content has a negative direct effect. The fresh ear weights direct effect is the highest among all characters. Mhoswa et al. (2016) and Priyanto et al. (2018), in their research also reported that fresh ear weight was a character with the highest direct effect to yield.

The relationship between number of seeds per row and yield is a mediation model. In the path analysis mediation, there is an intermediate variable between variables X and Z (Sudaryono, 2011). that variable can change the direction and magnitude of variable X's effect on variable Z. The total effect of variable X on Z in a mediation model, according to (Noviyanti et al., 2016), is the product of the X-Y and Y-Z pathways. This research reveals that the number of seeds per row affects yield through fresh ear weight. Figure 2 shows that the number of seeds per row's indirect effect to yield is 0.298, and the total effect of number of seeds per row to yield is 0.269.

Character shelling percentage shows an exciting finding. The relationship between shelling percentage and yield is the combination of multiple regression and mediation regression models (Sarwono, 2011). Character shelling percentage, besides having a direct effect to yield, also has an indirect effect through fresh ear weights. Figure 2 explains that the shelling percentage direct and indirect effect to yield is 0.214 and 0.468, respectively. Shelling percentage total effect to yield is 0.637.

It is effective to implement indirect selection for high-yield maize hybrid through shelling percentage and fresh ear weight. It can be inferred from coefficient correlation and direct effect value of shelling percentage and fresh ear weight to yield is almost equal. The direct effect and correlation coefficient of shelling percentage to yield is 0.637 dan 0.687. It is similar to Efendi et al. (2016) that state shelling percentage can be used in the maize yield indirect selection during drought conditions. The high-value direct effect and coefficient correlation of fresh ear weight to yield (0.903 dan 0.955) indicate that character is effective at the indirect selection. In indirect selection, selection will be effective when the coefficient correlation and direct effect of targeted selection are almost equal.

Conclusion

Agronomic characteristics with high heritability genetic variability are plant height, ear height, leaf angle, leaf length, ear diameter, and 1000 seeds weight. The characteristics with the highest yield effect are fresh ear weight and shelling percentage

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Growth optimization of white turmeric (*Curcuma zedoaria)* plantlets with growth regulators gibberellins

Abstract. The addition of cytokinin to the multiplication of white turmeric (*Curcuma zedoaria*) seedlings in vitro proved effective in shoot multiplication, but the addition of high cytokinin concentrations could cause stunted shoots and stunted growth. The addition of the hormone gibberellins (GA_3) is often used in tissue culture for shoot elongation, so increasing the viability of plantlets. The experiment aims to determine the effect of GA_3 and obtain the best concentrations of GA_3 consisting of 0.50, 1, and 1.5 ppm GA_3 on optimizing the growth of zedoary plantlets. The experimental parameters included the number of shoots, roots, leaves, plantlet height, and chlorophyll content observed at 6 WAP after subculturing. The results showed that giving 1 ppm GA_3 had the best effect on optimizing plantlets' growth, namely the growth component of the number of shoots and plantlets' height.

Keywords: *Curcuma zedoaria* · Plantlets height · Thidiazuron

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Introduction

White turmeric (*Curcuma zedoaria*) is an herbal plant of the Zingiberaceae family that has the potential to be cultivated. The content of sesquiterpenes, curcuminoids, and ethyl p-methoxycinnamate in white turmeric has cytotoxic activity against cancer and tumor cells by inhibiting cell proliferation and cell colony formation (Sagita et al., 2022). Therefore, white turmeric is widely used as an industrial raw material, especially in the biopharmaceutical industry.

In 2021, the production of the rhizome plant found, one of which is white turmeric has decreased to 203.74 tons, which previously reached 213.39 tons in 2020 (Statistics Indonesia, 2021). The decline in production certainly impacted the supply of industrial raw materials. According to Salim & Munadi (2017) there are 112 traditional medicine industries and 828 small traditional medicine businesses in Indonesia, 94% of these industrial raw materials come from within the country. Still, the supply of these industrial raw materials cannot be fulfilled due to the limited availability of simplicia.

The limited availability of white turmeric rhizome can be caused by harvesting the rhizome, which can only be done once a year so that the availability of simplicia and seed sources for the cultivation of white turmeric is increasingly limited (Puspita et al. 2019).

One way to overcome this problem is through tissue culture techniques. Tissue culture is a technique for isolating plant parts in the form of cells, tissues, organs, protoplasm, and other parts, which are cultured aseptically in a medium containing several nutrients (Ziraluo, 2021). This technique allows seed production to be done quickly and produces large quantities of seeds (Fong & Sani, 2019). According to Ziraluo (2021) seed production through tissue culture techniques is carried out under more controlled environmental conditions so that the seeds produced will be free from disease attacks.

The success of the growth and development of an explant through tissue culture techniques is influenced by the components contained in the culture media. Plant Growth Regulator (PGR) are one of the media components that affect the morphogenesis and organogenesis activity of a cell, besides that, the addition of ZPT affects the growth rate of tissue until it forms a complete plant (Rahman et al. 2021).

Growth regulators commonly used in seed production through tissue culture are cytokinins. Thidiazuron (TDZ) is a type of cytokinin most often used in seedling propagation through tissue culture because it accelerates cell division (Kholifah et al. 2022). However, giving TDZ in high concentrations can cause short shoots and abnormal growth because it affects the morphogenesis and organogenesis of an explant (Deepa et al. 2018).

Stem elongation occurs due to the process of cell division, elongation, and enlargement of cells in the stem tip meristem, resulting in an increase in height. The stem elongation activity can be induced by administering the growth regulator gibberellins (GA_3) (Bagale et al. 2022). Therefore, giving GA_3 can be used to optimize the growth of stunted shoots due to the addition of TDZ in high concentrations.

The effect of GA_3 on optimizing the growth of white turmeric through tissue culture has not been widely carried out. Therefore, this research was conducted to determine the effect and obtain the best concentration of GA_3 for optimizing the growth of white turmeric (*Curcuma zedoaria*) plantlets.

Materials and Methods

The research was conducted at the Tissue Culture Laboratory of the Agrotechnology Study Program, Faculty of Agriculture, Padjadjaran University. The research was conducted from November 2020 - April 2021.

Media preparation. The media used were Murashige and Skoog's media consisting of 30 g/L sugar, 2 g/L gelzan, and gibberellin growth regulator consisting of 0.50 ppm, 1 ppm, and 1.5 ppm.

Planting material and sterilization. The turmeric rhizome was obtained from the Biopharmaca Cultivation Conservation Unit of the Tropical Biopharmaceutical Center Institute for Research and Community Service, Bogor Agricultural University (IPB). The sterilization of white turmeric shoots was done twice, namely outside the Laminar Air Flow (LAF) and inside the LAF. Intersection white shoots are washed using running water until there is no dirt attached to the shoots. Shoots were soaked in

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detergent solution for 10 minutes and rinsed using sterile aquadest until clean. The shoots were soaked in a fungicide solution of 0.6 g/100 mL and bactericidal 0.1 g/100 mL for 24 hours and rinsed using sterile distilled water until clean.

Sterilization was continued in LAF by soaking the shoots in 70% alcohol for 5 minutes and rinsing with sterile distilled water three times; then, the shoots were soaked in 20% Clorox solution for 15 minutes and rinsed using sterile distilled water three times. The shoots were soaked again in 10% Clorox solution for 15 minutes and rinsed with sterile distilled water three times, then, the shoots were soaked in 0.1% $HgCl_2$ solution for 10 minutes, then rinsed using sterile distilled water until clean.

Planting. The explants used were the best plantlets aged 6 WAP with the highest shoot multiplication rate, namely those grown on TDZ media with a concentration of 0.3 ppm TDZ but with short plantlets with a height of 3.65 cm. Plantlets that are 6 WAP on TDZ media subcultured on GA_3 treatment media to see the optimization of the growth of white turmeric plantlets.

Data analysis. Analysis of the results of the study was carried out descriptively.

codes	GA_3 treatments
A	Without Plant Growth Regulator (Control)
B	0.50 ppm GA_3
C	1 ppm GA_3
D	1.5 ppm GA_3

Parameters observed included the number of shoots, roots, leaves, plantlet height, and total chlorophyll content, which were observed at 6 WAP after being subcultured.

Results and Discussion

The number of shoots. High shoot multiplication is an indicator of successful seedling production through tissue culture. One factor that affects the shoot multiplication rate is the use of appropriate growth regulators (Kajla et al. 2018).

Based on the research results, the addition of GA_3 growth regulator with several concentrations had an effect on the increase in the number of shoots at 6 WAP. Table 1 shows that treatment C, namely adding 1 ppm GA_3, has better potential in optimizing the formation of shoots, namely 2.22 pods, compared to treatments B (0.50 ppm GA_3) and D (1.5 ppm GA_3).

According to Rajagukguk et al. (2018) the ability of GA_3 to induce budding is due to the fact that GA_3 can act as a substitute for auxin in inducing shoot formation through cell division activity. Giving GA_3 can also increase the sugar content in plants to trigger growth which is used in the respiration process so that energy is formed and stimulates cell division (Asra et al. 2020)

Another thing that affects the increase in the number of shoots on GA_3 media is that the formation of meristemoids can cause on plantlets due to the use of the initial media, namely TDZ, for shoot multiplication so that the administration of GA_3 into the culture media affects the development and elongation of shoots (Rajagukguk et al. 2018).

The number of roots. Roots are an important part of a plant because they function to absorb water and minerals needed for plant growth. Roots are an important part of a plant because they function to absorb water and minerals needed for plant growth.

Table 1 shows that treatment C, namely 1 ppm GA_3, has better potential in root formation than other treatments. The number of roots formed in treatment C was 11.11. This condition is in accordance with the research of Padrón et al. (2020) on *Alpinia purpurata* plants which showed that giving GA_3 could increase the number of roots but not significantly different from the control treatment.

According to Triani et al. (2020) addition of gibberellins can stimulate the formation of roots due to the formation of proteolytic enzymes that can release tryptophan, namely the precursor of auxin so that gibberellins can increase the auxin content, which can induce rooting.

The number of leaves. Leaves are plant organs involved in several plant physiological activities such as photosynthesis, respiration, transpiration, synthesis, and provision of growth regulators. According to Novianto & Setiawan (2019), the number of leaves affects plant development; the more leaves that are formed, the more light that is captured by the leaves so that the process of photosynthesis will be faster. Based on the results of the study giving 1 ppm GA_3 showed a better effect than other treatments in leaf formation, with a total of 5.44 leaves.

Table 1. Number of Shoots, Number of Roots, Number of Leaves and Plantlet Height in GA_3 Media at 6 WAP

Treatments	Shoots number	Root number	Leaf number	Plantlet height (cm)
A = No treatment (control)	1.00	10.89	4.22	7.86
B = 0.50 ppm GA_3	1.55	6.55	3.78	9.06
C = 1 ppm GA_3	2.22	11.11	5.44	13.13
D = 1.5 ppm GA_3	1.66	7.33	4.33	9.50

Table 2. Leaf Chlorophyll Content in GA3 Media at 6 MST

Treatments	Chlorophyll content (mg/g) 6 WAP
A = No treatment (control)	0.66
B = 0.50 ppm GA_3	0.72
C = 1 ppm GA_3	0.57
D = 1.5 ppm GA_3	0.60

According to Farida & Rohaeni (2019) the formation of leaves due to the addition of the GA_3 hormone is due to the fact that the gibberellin hormone can stimulate cell division activity and increase phloem and xylem growth, so with this activity, the growth of the number of leaves increases.

Plantlet height. The increase in plant height is one indicator of plant vegetative growth. According to Do Vale et al. (2019), the height of plantlets resulting from better micropropagation when acclimatized is around 5cm – 15 cm, with a survival rate of up to 97%.

Based on the study's results, the addition of GA_3 with various concentrations affected the increase in plantlet height. Table 1. shows that treatment 1 ppm GA_3 has better potential in increasing plantlet height than other treatments. This is in accordance with the research of Behera et al. (2018), addition of 1 ppm GA_3 to Hedychium coronarium plants with stunted plantlet conditions due to too high TDZ concentrations showed the highest plantlet height compared to other treatments with a height of 5.2 cm at 4 WAP after subculture from 0.8 ppm TDZ media.

According to Noor et al. (2017) GA_3 can increase plant growth activities, such as stimulating cell division activity and cell elongation in stem meristems, so giving GA_3 can stimulate internode and stem elongation. The activity of cell elongation by GA_3 can be caused by increased cell wall plasticity followed by hydrolysis of starch into sugar. As a result, it can reduce water potential and allow water to enter cells, thus encouraging cells to develop (Rahman et al. 2019).

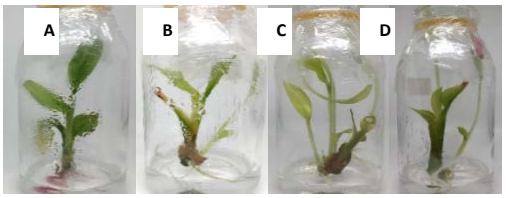

Figure 1. Plantlets Appearance for Each Treatment Age 6 WAP (A) 0 ppm GA_3 (B) 0.50 ppm GA_3 (C) 1 ppm GA_3 (D) 1.5 ppm GA_3

Total Chlorophyll Content. Chlorophyll is the most critical pigment associated with photosynthesis, absorbing energy from light, which is then used to convert carbon dioxide into carbohydrates (Zhao & Yaxin, 2014).

Table 2 shows that the 0.50 ppm GA_3 treatment has good potential in chlorophyll content compared to other treatments, which is 0.72 mg/g. According to Wen et al. (2018) GA_3 can increase the concentration of leaf chlorophyll, i.e., by increasing the number and size of chloroplasts and increasing plastid ultrastructural morphogenesis.

GA_3 influences plant biomass, especially in the vegetative parts, through the promotion of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein synthesis, ribose, and polyribosomes doubling, in addition to increasing enzyme activity and increasing membrane permeability which can facilitate absorption and use of mineral nutrition and transport of photosynthates (Miceli et al. 2019).

Wen et al. (2018) stated that the administration of exogenous GA_3 can affect source and sink relationships, including the strength of sources and sinks during the assimilation and partitioning of carbohydrates. The source is an organ or plant tissue that produces or exports photosynthate, while the sink is a photosynthate recipient (Mastur, 2015). Giving gibberellins can increase the power of the source by increasing the efficiency of photosynthesis and the energy of the sink by distributing photosynthetic assimilate.

Conclusion

Giving 1 ppm GA_3 gave the best effect for optimizing plantlet growth as indicated by certain variables of the number of shoots and plantlet height.

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The effect of herbicide glufosinate ammonium 150 g/L dose on several weeds and potatoes (*Solanum tuberosum*, L.) yield

Abstract. In the last three years, potato production in Indonesia fluctuated every year. One of the factors that cause low productivity of potatoes is weed. The presence of weeds in potato planting areas can inhibit plant growth and affect potato yields. The aim of this study is to determine the effectiveness of herbicide glufosinate ammonium 150 g/L to control common weeds in potato plants. This research was conducted in a farmer field in Lebak Muncang Village, Ciwidey District, Bandung Regency, West Java Province. The experimental design used was a randomized block design with six treatments and four replications. Weed control using the herbicide glufosinate ammonium 150 g/L at a dose of 2.75– 4.50 L/ha was completely (100%) controlled *Eleusine indica, Galinsoga parviflora, Amaranthus spinosus, Richardia brasiliensis,* and total weeds for up to 6 weeks after application without causing symptoms of poisoning and could increase the number of potato tuber per plant and yield of potato per plot.

Keywords: Weed · Glufosinate Ammonium · Herbicide · Potato

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Kurniadie D, Sumekar Y, Valent C. 2023. The effect of herbicide glufosinate ammonium 150 g/L dose on several weeds and potatoes (*Solanum tuberosum*, L.) yield. Jurnal Kultivasi, 22(1): 42-47

Introduction

Potato (*Solanum tuberosum* L.) is one of the root crops which is a source of carbohydrates that is widely consumed by most people in Indonesia. Potatoes have a fairly complete nutritional value and can be used as a substitute for rice (Robertson et al., 2018; Górska-Warsewicz, 2021). According to the Statistics Indonesia (2020) potato production in 2018 was 1,284,762 tons, in 2019 it increased by 1,314,657 tons, but in 2020 it decreased by 1,282,768 tons. Potato production in Indonesia each year has been recorded to have fluctuated in the last three years. The decrease of potato crop production is caused by various factors, one of which is the presence of weeds.

Weeds are very important in crop production, due to weeds can reduce the quality and quantity of the crops (Prayogo et al., 2017). According to Rao (2000) and Nurmala (2015), weeds can reduce crop yields by 10 to 25%. The presence of weeds on crops can reduce crop yields from 20 to 80% (Umiyati & Kurniadie, 2018). Weed control needs to be carried out to avoid a decrease in quality and quantity of crops (Bravo & Sembayang, 2020). Herbicides are chemical compounds used to inhibit growth and control weeds. The advantage of weed control using herbicides are more effective and efficient in terms of cost, time and labor as compared to other control methods (Lolitasari & Saifuddin, 2019).

Glufosinate ammonium 150 g/L works by inhibiting the synthesis of glutamine from glutamate, which is required for ammonia detoxification which causes ammonia to increase so that it reaches toxic levels in chloroplasts in leaf tissue (Takano & Dayan, 2020; Umiyati & Kurniadie, 2018). Glufosinate ammonium 150 g/L can control both broad leaf and grass weeds in fruit, rubber and oil palm plantations (Zhang et al., 2014; Ruzlan & Hamdani, 2020; Wibawa et al., 2009).

This study was to determine the effectiveness of the herbicide glufosinate ammonium 150 g/L to control weeds, growth and yield of potato.

Materials and Methods

This research was conducted from September 2021 to January 2022. in a farmer's field in Lebak Muncang Village, Ciwidey District, Bandung Regency, West Java Province at an altitude of 1200 meters above the sea level with the climate type C2 according to Oldeman.

The research design used was Randomized Block Design that consisted of six treatments and each treatment was replicated four times, so that there were 24 experimental units. Herbicide treatment consists of doses of (A) 2.75 L/ha, (B) 3.00 L/ha, (C) 3.75 L/ha, (D) 4.50 L/ha, (E) manual weeding and (F) control. The spray volume was calibrated at 400 L $Ha^{-1.}$ The application of the tested herbicide is carried out in the morning at 08.00 a.m when the weather is sunny and the wind speed is low at 23 **°C**. it was carried out only once before the planting period. Potato tuberss were planted by digging the soil to a depth of 3-5 cm, each hole was filled with one tuber with a row spacing of 30 x 70 cm. Fertilizing was applied twice, the first fertilizer was applied at 3-4 days before planting and the second fertilizing is carried out at 6 WAP (Weeks After Planting).

The observation variables consist of supporting observations (vegetation analysis using the formula relative density + relative dominance + relative frequency divided by three, environmental conditions and also phytotoxicity of potato which was carried out visually on the entire plant population at 1, 2, 3 weeks after planting), whereas the main observations were total weed dry weight on 3 and 6 Weeks After Application (WAA) in each treatment plot using 2 quadrat with the size of 50 cm x 50 cm, plant growth, yield components and yields of potato. The observations of plant height were carried out by taking 20 samples of potato plants at random at 3 and 6 WAP.

Results and Discussions

During the experiment, the amount of rainfall ranged from 60-520 mm/month, while the optimal rainfall was 200-300 mm/month (Hanan et al., 2015). The average humidity ranges from 74-82% and the temperature ranges from 23.4-24.3 ^{o}C. Temperature and humidity can affect the growth of potatoes, if the temperature is more than 30 °C then the growth of potatoes will be inhibited. The appropriate humidity for potato is 80% to 90% (Suryana, 2013). The range of Temperature and humidity were suitable for growing potato.

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The results of the vegetation analysis on the land before the experiment was carried out showed that there were 4 species of broadleaf weeds, 2 species of grass weeds and 1 species of sedge weed.

Table 1. Vegetation analysis

No	Weed species	Group of weeds	SDR (%)
1.	*Eleusine indica*	grasses	23.06
2.	*Galinsoga parviflora*	Broadleaf	19.20
3.	*Amarhantus spinosus*	Broadleaf	18.48
4.	*Richardia brasiliensis*	Broadleaf	13.73
5.	*Portulaca oleracea*	Broadleaf	9.56
6.	*Cyperus rotundus*	sedge	8.81
7.	*Cynodon dactylon*	grasses	7.17
	Total		**100.00**

There were four dominant weeds species, namely one grass weed such as *Eleusine indica* with SDR value by 23.06% and three broadleaf weeds. *Galinsoga parviflora; Amaranthus spinosus;* and *Richardia brasiliensis* with values were 19.20; 18.48; and 13.73%, respectively. Weeds that have an SDR value above 10% are called dominant weeds.

Phytotoxicity. Phytotoxicity is the percentage of the degree of poisoning of cultivated plants caused by herbicides. Based on the data presented in Table 2, it shows that there were no symptoms of poisoning due to the influence of the herbicide glufosinate ammonium 150 g/L on the growth of potato plants in 1, 2, and 3 WAP. The toxicity with a score of zero, indicates there is no poisoning or the percentage was only 0-5%.

Table 2. Phytotoxicity

Treatments	Dose (L/ha)	Toxicity Rate		
		1 WAP	2 WAP	3 WAP
A	2.75	0	0	0
B	3.00	0	0	0
C	3.75	0	0	0
D	4.50	0	0	0
E	manual	0	0	0
F	control	0	0	0

WAP: week after planting

Dry Weight of *Eleusine indica*. *Eleusine indica* weeds were the most dominant weeds in experimental fields. Based on the data in Table 3, the average of dry weight of *Eleusine indica* weeds at 3 WAA (Weeks After Application) and 6 WAA showed that the herbicide treatment of glufosinate ammonium 150 g/L at a dose of 2.75 ; 3,00 ; 3.75 and 4.50 L/ha showed a significantly different as compared to the control treatment. Total physiological characteristics included chlorophyll, survival, tillers, fresh weight, and dry weight of *Eleusine indica* weeds can (100%) suppressed by glufosinate ammonium and triclopyr at 2 to 8-fold of the recommended dose (Tampubolon *et al*., 2019).

Table 3. Average Dry Weight of *Eleusine indica*

Treatments	Dose (L/ha)	Average Dry Weight of *Eleusine indica*	
		3 WAA	6 WAA
A	2.75	0.44 a	2.98 c
B	3.00	0.23 a	1.31 b
C	3.75	0.42 a	0.94 ab
D	4.50	0.05 a	0.63 a
E	manual	2.88 b	6.50 d
F	control	5.39 c	10.73 e

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

Dry Weight of *Amaranthus spinosus*. Herbicide treatment of Ammonium Glufosinate 150 g/L at a dose from 2.75 until 4.50 L/ha indicate that the herbicide were effective in controlling *Amaranthus spinosus* weeds up to 6 WAA as compared to the control treatment. This was because the entire dose level of the herbicide Ammonium Glufosinate 150 g/L has been absorbed by the leaves so that it enters the site of action which causes weeds to die (Hastuti *et al*., 2017). This can happen because the leaf morphology of the broad leaf group weeds has a growing point of the apical meristem which is very sensitive to herbicides. The wide leaf surface causes the droplets of the applied herbicide Ammonium Glufosinate to be absorbed properly so that it is more effective for controlling *Amaranthus spinosus* weeds as compared to manual weeding.

Table 4. Average Dry Weight of *Amaranthus spinosus*

Treatments	Dose (L/ha)	Average Dry Weight of *Amaranthus spinosus*	
		3 WAA	6 WAA
A	2.75	0.24 a	2.04 c
B	3.00	0.0 a	1.53 abc
C	3.75	0.0 a	0.77 a
D	4.50	0.0 a	0.86 ab
E	manual	2.38 b	4.60 d
F	control	5.46 c	8.49 e

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

Dry Weight of *Galinsoga parviflora.* Based on the data in Table 5 the average of dry weight value of *Galinsoga parviflora*, shows that the control treatment has the highest average dry weight compared to the Ammonium glufosinate herbicide treatment at each dose. This is because *Galinsoga parviflora* comes from the family Astraceae whose life cycle is annual weeds. According to Umiyati et al., (2015) annual weeds are weeds that have a life cycle lasting for one year starting from germination, production, to death. Herbicide treatment of glufosinate ammonium at all dose levels is effective in suppressing the growth of *Galinsoga parviflora.*

Table 5. Average Dry Weight of *Galinsoga parviflora*

Treatments	Dose (L/ha)	Average Dry Weight of *Galinsoga parviflora*	
		3 WAA	6 WAA
A	2.75	0.84 a	2.33 b
B	3.00	0.0 a	0.51 a
C	3.75	0.0 a	0.25 a
D	4.50	0.0 a	0.41 a
E	manual	1.82 b	6.97 c
F	control	11.49 c	12.67 d

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

Dry Weight of *Richardia brasiliensis.* Based on Table 6, it shows that at observations of 3 and 6 WAA, the treatment of glufosinate ammonium 150 g/L at a dose of 2.75 L/ha until 4.50 L/ha gave a lower average of *Richardia brasiliensis dry weight* at 3 and 6 WAA and significantly different as compared control treatment. According to Umiyati & Kurniadie (2018), application of the herbicide glufosinate ammonium 150 g/L causes the synthesis of glutamine from glutamate to be inhibited, causing ammonia to increase which causes toxic levels in chloroplasts in leaf tissues, so the photosynthesis will stop and weeds will die.

Table 6. Average Dry Weight of *Richardia brasiliensis*

Treatments	Dose (L/ha)	Average Dry Weight of *Richardia brasiliensis*	
		3 WAA	6 WAA
A	2.75	0.07 a	1.03 c
B	3.00	0.0 a	0.54 ab
C	3.75	0.0 a	0.0 a
D	4.50	0.0 a	0.35 a
E	manual	0.84 b	2.28 c
F	control	4.33 c	5.18 d

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

Dry Weight of Other Weeds. Based on Table 7, it shows that at observations of 3 and 6 WAA, the average dry weight value of other weeds at the herbicide glufosinate ammonium dose 2.75 until 4.50 L/ha were higher as compared to control treatment. This shows that application of herbicide glufosinate ammonium 150 g/L from 2.75 until 4.50 L/ha was effective to control other weed species. According to Sihombing (2020), herbicide glufosinate ammonium 150 g/L is non-selective and broad-spectrum and can control not only broad leaf but also grass and sedge weeds.

Table 7. Average Dry Weight of Other Weeds

Treatments	Dose (L/ha)	Average Dry Weight of Other Weeds	
		3 WAA	6 WAA
A	2.75	1.24 a	3.80 bc
B	3.00	0.14 a	2.66 abc
C	3.75	0.17 a	2.38 ab
D	4.50	0,14 a	2.18 a
E	manual	2.86 b	7.04 d
F	control	15.28 c	19.47 e

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

The average of total weed dry weight was presented in Table 8. Table 8 showed that at 3 and 6 WAA the treatment of glufosinate ammonium 150 g/L with a dose of 2.75 g/L ; 3.00 g/L ; 3.75

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g/L ; 4.50 g/L had a lower average of total dry weight than control treatment. This was probably due to systemic type of herbicide glufosinate ammonium 150 g/L which can be translocated to all parts of the weed which causes the weed to be suppressed so that the weed can be controlled properly.

Table 8. Total Dry Weight of Weeds

Treatments	Dose (L/ha)	Total Dry Weight of Weeds	
		3 WAA	6 WAA
A	2.75	2.84 b	12.17 c
B	3.00	0.38 a	6.55 b
C	3.75	0.21 a	4.34 a
D	4.50	0.18 a	4.44 a
E	manual	10.78 c	27.40 d
F	control	41.96 d	56.55 e

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAA=week after herbicide application

Plant Height Table 9 showed that at the observations 3 and 6 WAP, the treatment of herbicide glufosinate ammonium 150 g/L at a dose of 2.75 until 4.50 L/ha have higher average of plant height and significantly different than control treatment. This proves that all herbicide treatments doses of 2.75 L/ha to 4.50 L/ha are able to effectively suppress weeds so that they can affect the growth of potato plants as compared to manual weeding treatment.

Table 9. Average Potato Plant Height

Treatments	Dose (L/ha)	Average Potato Plant Height	
		3 WAP	6 WAP
A	2.75	6.06 b	29.12 abc
B	3.00	5.99 b	26.54 ab
C	3.75	5.75 b	31.27 bc
D	4.50	6.00 b	30.26 abc
E	manual	6.48 b	33.13 c
F	control	4.56 a	24.60 a

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test. WAP: week after planting

Amount, number of potatoes per plant and yield per plot. The observations on the number of potatoes and weight of potatoes per plant and per plot were carried out at harvest time, which was 90 DAP (Days After Planting). Table 10 showed that the treatment of the herbicide glufosinate ammonium 150 g/L at a dose of 2.75 until 4.50 L/ha had an average value of the number of potatoes per plant, weight of potatoes per plant and weight of potatoes per plot higher than the control treatment. This is because the herbicide glufosinate ammonium 150 g/L can control the growth of *Eleusine indica, Amaranthus spinosus, Galinsoga parviflora, Richardia brasiliensis* and other weeds from 3 until 6 DAA, so the competition between potato and weeds was reduced. The lower the level of weed competition, the more optimal the main crop to grow, on the other hand, if the weed competition is high, the main crop will find it difficult to find nutrients for crop growth (Latifa et al., 2015).

Table 10. Average Amount of Potatoes and Weight of Potatoes

Treatments	Dose (L/ha)	Number of Potatoes Per Plant	Dry Weight Per Plant (gram)	Potato Weight Per Plot (kg)
A	2.75	9.30 b	395.46 bc	19.75 bc
B	3.00	10.25 c	408.58 c	20.42 c
C	3.75	10.37 c	394.66 bc	19.72 bc
D	4.50	10.50 c	409.36 c	20.42 c
E	manual	9.23 b	382.32 b	19.07 b
F	control	6.98 a	315.66 a	15.77 a

Description: The average value of the treatment followed by the same letter in the same column indicates no significant difference based on the 5% LSD Test.

Conclusion

Weed control using the herbicide glufosinate ammonium 150 g/L at a dose of 2.75 – 4.50 L/ha was completely (100%) controlled *Eleusine indica, Galinsoga parviflora, Amaranthus spinosus, Richardia brasiliensis* and total weeds for up to 6 weeks after application without causing symptoms of poisoning and can increase number of potato tuber per plant and yield of potato per plot.

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Growth and yield pattern of microgreen under different types of artificial lighting

Abstract. Microgreen is a popular food product that is interesting to study and can be produced in the building with the support of artificial light, especially in terms of smart lights. This study aims to analyze the growth and yield response of red amaranth, red radish, and coriander microgreens under different types of light color treatment from smart lights. The study was conducted in August 2022 at the microgreen culture room, Department of Agronomy, Universitas Padjadjaran, Indonesia, using a completely randomized design with two factors, namely three levels of plant species and five levels of smart light color. The results showed differences in seed viability, first-day count, and the day the cotyledons were open among three microgreen species. Seed growth into microgreens had the same pattern, namely linear and positive, even in the light absence condition as the evidence of etiolation occurrence leading to the production of thin and yellow pale color of microgreens. The difference in light color is specific to each type of plant. Red radishes thrived in all colors, although red light tended to do better. On the other hand, red amaranth was inhibited in red light and coriander in white light. Blue light is strongly recommended for increasing red amaranth and coriander microgreens yields.

Keywords: Red amaranth · Red radish · Coriander · Smart lamp · Microgreen

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Introduction

Microgreen is a food ingredient that is produced simply as a form of continued development of sprouts (Verlinden, 2020) with a crispy texture and attractive colors and is easily found in modern food, such as salads and sandwiches (Renna et al., 2017; Turner et al., 2020). It is a new popular culinary trend in recent years, especially among urban communities (Stoleru et al., 2016; Riggio et al., 2019a), that is used as a garnish to increase the aesthetic value of food appearance.

In addition to aesthetic aspects, microgreen also offers health benefits since it contains vitamins, minerals, carotene, and antioxidant compounds (Xiao et al., 2012; 2015; 2016; 2019). Some plants are new idols of microgreen, e.g., red radish, red amaranth, and coriander. Red radish microgreens are rich in anthocyanins (Zhang et al., 2019). Red amaranth contains higher carotenoid lutein/zeaxanthin (Xiao et al., 2012). Coriander microgreen contains more phenol and terpene than its vegetable form (Oruna-Concha, 2017). It is strongly stated that microgreen is functional food to maintain good health (Choe et al., 2018).

The attractiveness of microgreens in terms of aesthetics and health increases people's interest in producing microgreens. The production of this microgreen can be carried out both outdoors and indoors. The consequence of producing microgreens indoors is the need for artificial irradiation support to replace the sunlight. The development of technology has now allowed interaction between the field of artificial intelligence and agriculture, such as smart artificial lamps. The advantage of using smart lamps is the ease of setting the on/off and color types automatically anytime, anywhere.

The arrangement of these lamps is interesting to study because the light is one of the abiotic factors determining plant growth and development (Gupta et al., 2018). Lamp selection is more advisable to use light-emitting diodes than fluorescent tubes with energy-saving considerations (Shukla et al., 2017). Previous research has reviewed the influence of artificial LED lamp colors on microgreens' growth, yield, and phytochemical content (Putri et al., 2022). But more specific research on new microgreen idols, such as red radish, red amaranth, and coriander, is still limited. This study aims to analyze the growth response and microgreen results of red radish, red amaranth, and coriander in response to different light colors of smart lamps.

Materials and Methods

This experiment was conducted at the microgreen culture room, Department of Agronomy, Universitas Padjadjaran, in August 2022. The microgreen planting material used is the seeds of the red amaranth (*Amaranthus cruentus* L.), red radish (*Raphanus sativus* L.), and coriander (*Coriandrum sativum* L.). Seeds were obtained from the online market. The germination medium of vermiculite for about 8.5 g is placed in a plastic bowl planting container and arranged according to the experimental design.

This study used an experimental design in the form of a Factorial Randomized Completely Blok Design consisting of two factors: the type of microgreen and the color of the lamp. The first factor, namely the type of microgreen, consists of three levels, i.e., red amaranth, red radish, and coriander. In contrast, the second factor, namely the lamp's color, consists of five levels, i.e., white, red, blue, purple (a combination of red and blue), and dark conditions.

This study was composed of 15 combinations of treatments replicated 18 times to accommodate three tests in each destructive observation, and in total, there were six destructive observations.

This study used a multistage planting rack according to the illustration in Figure 1A. At each level, smart lights are installed on the roof at a distance of 30 cm from the surface base of each shelf. Smart lamps can be adjusted the color type on smartphones with the help of wi-fi. Using smart lights also makes it easier to set the length of the day/photoperiodic, namely 10 hours of day and 14 hours of dark, by using the scheduling feature so that the smart lights will turn on and out automatically according to the predetermined schedule.

On each shelf, the microgreen planting container is arranged in a circle according to the illustration in Figure 1B. Each circle has three types of microgreens with the same number of replications. This is intended to avoid variations in the irradiation intensity between the innermost circle close to the light source and the outermost circle further away from the light

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source. For dark treatment, planting containers are arranged and located in plastic boxes on the top shelf. The color of the lamp from the second layer up to the bottom in successive ways are as follows: red, purple, blue, and white (Figure 1B). The walls on each shelf are covered with black cloth to minimize the light bias from other treatments.

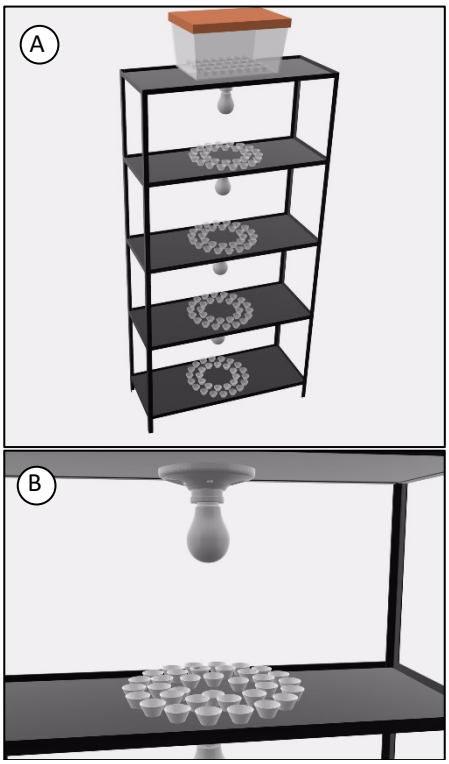

Figure 1. A) illustration of multi-storey racks and placement of smart lights, B) illustration of microgreen containers at each shelf level.

Microgreen planting is carried out by soaking the seeds in warm water for 8 hours before sowing. Post-soaking seeds are ready for sowing, with about 20 seeds in each planting container. Watering is carried out every two days with the sprayer. The volume of watering per planting container is 10 mL.

The observation was carried out on the variable of growth and yield. Growth improvement were represented by hypocotyl length and microgreen fresh weight that were carried out daily and destructively from the 2nd to the 7th day after germination. The hypocotyl length was measured using a mini ruler from the base of the growing medium to the base of the opening leaves. The fresh weight of microgreen was weighed using an analytical balance with an accuracy scale of 0.001 g. The increase in the hypocotyl length and the microgreen's fresh weight were presented in the form of line charts. Data on the microgreen hypocotyl length and fresh weight on the 7th day were tested by analysis of variance and Duncan's test on SPSS software. Other variables observed were seed viability, first-day count, first-day of leaves open, hypocotyl color, leaves color and shape; however, it was not followed by statistical analysis, only describe qualitatively.

Results and Discussion

Microgreen Growth. There were statistically differences of seed viability among tested genotypes, with the highest value in the type of red radish at 100%, red amaranth at 90%, and coriander at 75% (Table 1). Seed viability is defined as the seed ability to germinate normally under optimal conditions. The difference in seed viability could be caused by genotype factors and the actual condition of seed quality. Low viability is one of the indicators for seed quality deterioration. Improper seed storage is one of the factors causing the seed quality deterioration (Copeland & McDonald's, 2001).

Table 1. Seed viability, the first day of germination, and the first day of fully open leaves of microgreens.

Changer of Observations	Plant Genotype		
	Red Amaranth	Red Radish	Coriander
Seed viability	90%	100%	75%
First-day count	3 DAS	3 DAS	6 DAS
First-day of leaves open	5 DAG	4 DAG	7 DAG

Notes: The seed viability is obtained from the number of normal germinated seeds divided by the number of seed sowed in the container and expressed in percentage. DAS = day after sowing; DAG = days after germination

The genotype factor is also thought to be the cause of the difference in first-day count and first-day of leaves open. Red radish and red amaranth have similar first-day count, i.e., 3 days after sowing (DAS), while coriander takes a longer time to germinate, which is 6 DAS (Table

1). The next growth process is the lengthening of the hypocotyl and the opening of the leaves (Figure 2).

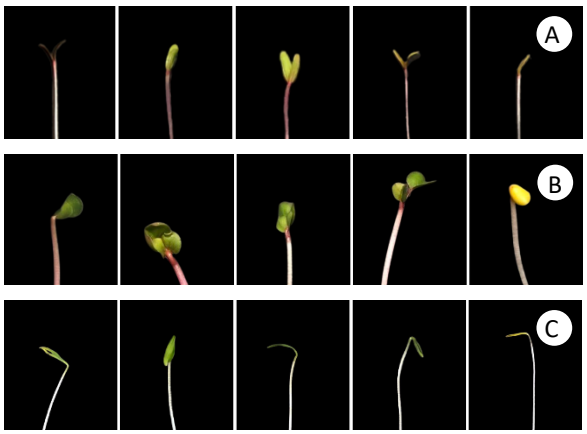

Figure 2. The color of hypocotyl and leaves of three microgreen species (A - red amaranth, B - red radish, C - coriander) at 7 DAG under different colors of artificial light, i.e. white, blue, purple, red, and dark (from left to right)

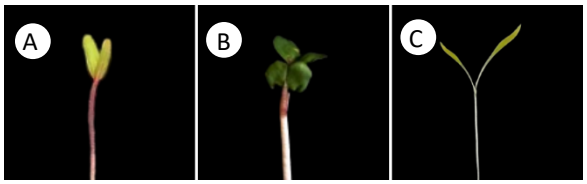

Figure 3. The leaf shape of three microgreen species, i.e., A) red amaranth, B) red radish, C) coriander.

The hypocotyl is the central part of microgreen that looks like a main stem. Hypocotyl is generally cylindrical, with colors varying depending on genotype and environmental factors. Red amaranth and red radish are reddish hypocotyls, while coriander is white. Ecological differences, in this case, artificial lighting treatment causes color differences, i.e., the dark caused pale yellow leaves, while lamp-treated microgreen displayed green color on red radish or light green in red amaranth and coriander (Figure 2).

The absence of light in dark treatment inhibits chlorophyll synthesis since chlorophyll becomes a green coloring agent on the leaves (Taiz & Zeiger, 2009). The longer a plant is exposed to the dark, the more etiolation observed, followed by a lower pigment content (Niroula et al., 2021).

Aside from hypocotyl, the leaf is also main counterpart of microgreen that is located at the tip of the hypocotyl growing point. The shape of the leaves is strongly influenced by genotype factors. The color of red amaranth, red radish, and coriander leaves is successively light green, dark green, and light green, respectively. The shape of these leaves is quite varied, namely oblong for red amaranth, obcordate for red radish, and linear for coriander (Figure 3). Red radish is determined as the fastest microgreen to reach fully open leaf condition, and then followed by red amaranth, and the slowest one is coriander (Table 1).

The results showed that there were linear and positive growth patterns of the three genotypes of microgreens under different artificial light treatments. It is proved by the increase in hypocotyl length and fresh weight of microgreens on the 7th day compared to the previous days (Figures 4 and 5).

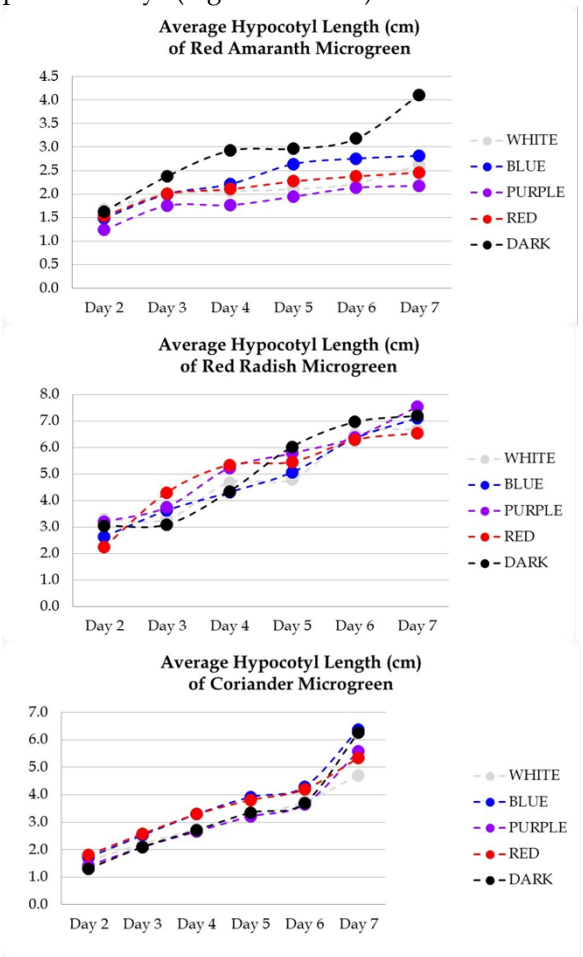

Figure 4. Daily (from day 2 to day 7) hypocotyl length (cm) of three microgreen types (red amaranth (top), red radish (middle), coriander (bottom)) under different light colors of artificial lamp.

The average of hypocotyl length gain in red amaranth microgreens illuminated by white light, blue light, purple light, red light and dark condition are as follows 0.37; 0.40; 0.31; 0.35; and

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0.59 cm per day, respectively (Figure 4 above). In the case of fresh weights, the average of this microgreen white light, blue light, purple light, red light and dark condition are as follows 1.2; 1.2; 1.0; 1.0; and 1.2 mg per day, respectively (Figure 5 above). In the dark treatment, a very rapid increase in the length of the hypocotyl on day 7, was not followed by an increase in its hypocotyl weight. This makes the red amaranth microgreen appearance in the dark is thinner due to intense etiolation.

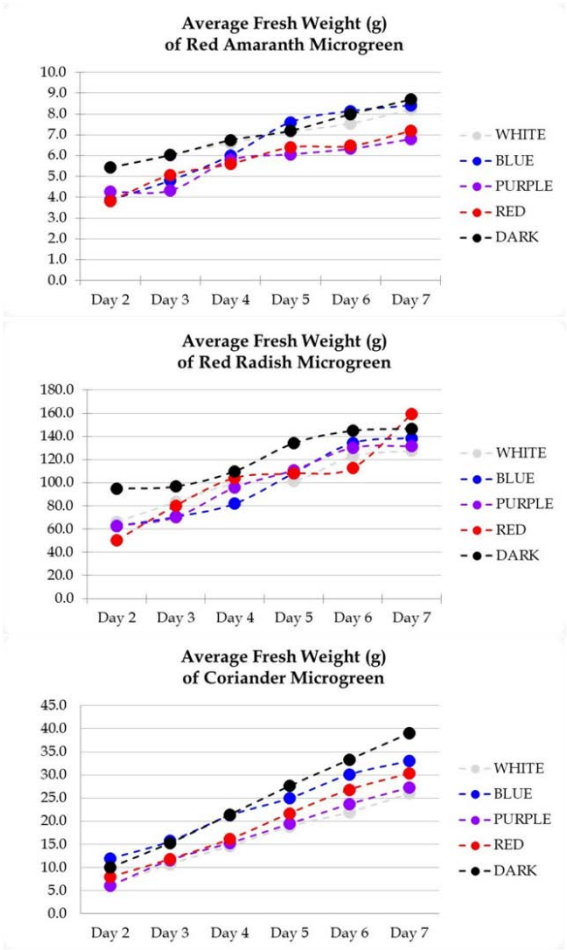

Figure 5. Daily (from day 2 to day 7) microgreen fresh weight (mg) of red amaranth (top), red radish (middle), coriander (bottom) under different light colors of artificial lamp.

In red radish, the mean of increase in hypocotyl length are 0.96; 1.01; 1.08; 0.93; and 1.03 cm per day, on white light, blue light, purple light, red light, and dark treatments, respectively (Figure 4 middle). This type of microgreen also experienced an increase in fresh weight by 18, 20, 19, 23, and 21 mg per day under white light, blue light, purple light, red light, and dark condition (Figure 5 middle).

The coriander microgreen response also showed an increase in hypocotyl length by 0.67; 0.9 1; 0.80; 0.76; and 0.89 cm per day under white light, blue light, purple light, red light and dark treatment, respectively (Figure 4 below). In parallel with the increase in the hypocotyl length, there was also an increase in microgreen fresh weight by 3.7; 4.7; 3.9; 4.3; and 5.6 mg per day due to white light, blue light, purple light, red light, and dark treatment (Figure 5 below).

Microgreen Yield Component. The microgreen yield component can be represented by microgreen fresh weight and hypocotyl length at 7 DAG. This early harvesting activity produces microgreen immature products with colors, sizes, and textures that are preferred compared to late harvesting at 14 DAG.

Table 2. Fresh weight of three types of microgreens at 7 days after germination (DAG) under different light colors of artificial lamp.

Treatment	Microgreen Weights (mg)
- Red Amaranth (*Amaranthus cruentus* L.) -	
White	8.2 b
Blue	8.4 b
Purple	6.8 a
Red	7.2 a
No lights	8.7 b
- Red Radish (*Raphanus sativus* L.) -	
White	127.8 a
Blue	138.5 a
Purple	131.7 a
Red	159.3 a
No lights	146.7 a
- Coriander (*Coriandrum sativum* L.) -	
White	26 a
Blue	33 ab
Purple	27.3 a
Red	30.3 ab
No lights	39 b

Note: The mean followed by the letter in the same column showed no significant difference based on Duncan's test at the level of 5 %.

There is a difference in microgreen fresh weights between genotypes with the highest value on red radish, while the smallest one in red amaranth. Light color treatment resulted in significant variations of fresh weight of red amaranth and coriander at 7 DAG. However, this is not significantly observed in red radish, although there is a tendency of superior result under red light irradiation (Table 2 and Table 3).

Dark-treated red amaranth has the highest fresh weight and hypocotyl length, as a result of a

severe etiolation compared to light-treated microgreen. That results is also similar to previous study by Zelenkov et al., (2019). In red amaranth, the irradiation of red and purple actually produces a short hypocotyl and light microgreen. However, our finding was in contrary to previous report on tomatoes (Kalaitzoglou et al., 2019) and some species of the Brassicaceae family (Signore et al., 2020).

Table 3. Hypocotyl length of three types of microgreens at 7 days after germination (DAG) under different light colors of artificial lamp.

Treatment	Length of hypocotyl (cm)
- Red Amaranth (*Amaranthus cruentus* L.) -	
White	2.62 b
Blue	2.8 b
Purple	2.18 a
Red	2.46 ab
No lights	4.11 c
- Red Radish (*Raphanus sativus* L.) -	
White	6.69 a
Blue	7.10 a
Purple	7.53 a
Red	6.53 a
No lights	7.21 a
- Coriander (*Coriandrum sativum* L.) -	
White	4.71 a
Blue	6.38 b
Purple	5.59 ab
Red	5.35 ab
No lights	6.26 b

Note: The mean followed by the letter in the same column showed no significant difference based on Duncan's test at the level of 5 %.

For the production of red amaranth microgreen, the treatment of white or blue lamps is recommended since they can produce the best microgreens with a good fresh weight and full color (reddish) hypocotyl, as a sign of the carotenoids-rich, in contrast to pale white color under no light treatment.

In the case of coriander, the provision of white light actually produces the lowest yield, while blue light produces the best fresh weight and length of the microgreen. Similar result was also reported by Signore et al., (2020) who observed the superiority of blue light over white light. Previous study by Park et al., (2010) proved the influence of blue light in the induction of plant biomass production. The increase in growth response that leads to an increase in yield is thought to be main positive influence of blue light, especially related to the amount of chlorophyll, as reported by Ouzounis et al., (2016). The results of this study also justify that there is an opportunity to optimize microgreen yields by knowing the specifications of the desired color light for each type of microgreen. Further research related to the influence of lamp types on the content of useful phytochemicals is interesting to study.

Conclusion

There are differences in viability, first-day count and first- day of the leaves open among three genotypes of microgreen. Seed growth into microgreens has the same pattern of linear and positive. In dark conditions, the intensity of etiolation increases so that the microgreen is thin and pale yellow. The light color is specific to each type of microgreen. Red radish microgreen can be produced well on all tested colors, although there is a better tendency to red light. On the other hand, red amaranth is actually hampered under red light and coriander under white light. Blue lamps are recommended for increased yields of red amaranth and coriander microgreens.

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Growth response of not-ready-to-distribute tea (*Camellia sinensis* (L) O. Kuntze) seedlings due to application of biofertilizer at various concentrations and intervals

Abstract. The plantation rejuvenation program makes the need for ready-to-distribute tea seedlings getting higher. Meanwhile, the nursery's seedlings are mostly not ready to distribute. This study aimed to determine the response of not-ready-distribute tea seedlings due to the application of biofertilizers at various concentrations and intervals. This experiment was carried out at Gambung Tea and Quinine Research Center Nursery, conducted from December 2021 to February 2022 at an altitude of 1,350 meters above sea level (asl). The experimental method used was a randomized block design with eight treatment combinations, namely: control (no fertilizer); urea fertilizer every two weeks; Biofertilizer 5 mL L^{-1} + interval once a week; Biofertilizer 5 mL L^{-1} + interval every two weeks; Biofertilizer 10 mL L^{-1} + break once a week; Biofertilizer 10 mL L^{-1} + interval every two weeks; Biofertilizer 15 mL L^{-1} + interval once a week; Biofertilizer 15 mL L^{-1} + interval every two weeks, all repeated four times. The experimental results showed that treatment of biological fertilizers influenced the parameter number of leaves and chlorophyll index. The application of biofertilizer with a concentration of 15 mL L^{-1} + interval of 2 weeks greatly influenced the parameters of leaf number and chlorophyll content index.

Keywords: Biofertilizer · Concentration · Interval · Not ready to distribute tea seedlings

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Introduction

The problem tea commodities face today in Indonesia is the yearly decline in tea production. Contrary, the high demand for tea products must be fulfilled. Statistics Indonesia noted that the volume of tea production in 2018 decreased by 4.11% from 2017, followed by a decline in export volume of 9.52% (Statistics Indonesia, 2020). Some factors have contributed to this, namely a decrease in the area of the plantation, the unproductive age of plants, and the plant population of fewer than 10,000 trees/ha (Fitria, 2016). One solution to these problems is by rejuvenating old tea plants through replanting. The rejuvenation of old tea plants and the increasing population requires the availability of quality seeds and adequate quantities (Suherman et al., 2015).

A suitable tea nursery is the first step to getting high-quality and sustainable tea plants. Salim et al. (1996) stated that the balanced development of roots, stems, and leaves characterized seedlings growth. With a survival percentage reached > 95% when transferred to the field. Tea nursery is expected to produce a success rate of 80% of tea seedling growth; the percentage of tea seedlings growth only reaches 40-50%, which causes a low percentage of seedlings ready for distribution (Wulansari *et al*., 2016). According to Suherman et al. (2015), the percentage of tea seedlings that are not ready for distribution is 40 - 50%. Characteristics of tea seedlings ready to distributed have a minimum height of 25 cm, a minimum number of leaves of 5-6 leaves, and 1 year old, while the seedlings that do not meet these criteria are included in the seedlings not ready to distributed (Suherman et al., 2015). The slow-growing rate of seedlings at the nursery causes the seedlings not to be ready for distribution. Several factors drive the high number of not-ready-to-distribute seedlings: inappropriate planting media, poor plant material, and unfulfilled nutrients (Wulansari *et al*., 2016). If one of the needed nutrients is not fulfilled, the plant metabolic processes will be inhibited, affecting plant growth and development (Salisbury & Ross, 1992). One of the efforts that can be made to improve the quality of tea seedlings that are not ready for distribution is fertilizing.

Generally, the not-ready-to-distribute tea seedlings are treated with urea fertilizer with a concentration of 2% until 1-year-old seedlings are ready for distribution (Naomi & Rahadi, 2019). Urea is easily dissolved, but the application of it on the surface can cause N loss to the air up to 40% of the applied N (Ramadhani et al., 2016). Using inorganic fertilizers should be balanced with organic fertilizers, one of which is using biological fertilizers. Biofertilizers are inoculants that contain active living organisms in solid or liquid form. When applied to seeds, plant surfaces, or soil can stimulate the growth of these plants (Vessey, 2003). Biofertilizers can accelerate plant growth because they contain nitrogen-fixing microorganisms (Prasetyo, 2018). Biological fertilizers can reduce the use of inorganic fertilizers to protect the environment from the impact of excessive use of inorganic fertilizers (El Salam, 2007). According to Enice et al. (2019), the right time for fertilizer application will increase plant growth and production. If the interval is too frequent, it can lead to too luxurious consumption, causing fertilizer wastage. On the other hand, the infrequent interval can lead to insufficient plant nutrient requirements (Rajak et al., 2016). This research was carried out to determine the effect of concentration and intervals of biological fertilizers to accelerate the growth of not ready-to-distribute tea seedlings.

Materials and Methods

This research was conducted at the Experimental Field of Tea and Quinine Research Center (PPTK) Gambung, at an altitude of 1,350 meters above sea level, Andisol soil order and climate type B according to the Schmidt-Ferguson classification, from December 2021 to February 2022. The materials used were GMB 7 clone tea seedlings aged eight months for the class C seed category or not ready to distribute, urea fertilizer, and biological fertilizers with the trademark Bion-Up that contains *Azotobacter* sp *Azospirillum* sp, *Pseudomonas* sp, *Acinetobacter* sp, *Penicillium* sp, Gibberellin Hormones, Auxin Hormones, Cytokinin Hormones based on analysis of UNPAD Soil Chemistry and Plant Nutrition Laboratory. The tools used were writing instruments, rulers, scales, a caliper, a hand sprayer, a chlorophyll meter with SPAD 502 type, and an oven.

The experimental method used was a randomized block design (RBD) with eight

treatment combinations. Each treatment was repeated 4 times so that there were 32 experimental units. Each experimental unit contained 5 plants, so the total number of plants was 160 plants. The treatments are arranged as follows: A = control (no fertilizer); B = urea fertilizer every two weeks; C = biofertilizer 5 mL L^{-1} + interval once a week; D = biofertilizer 5 mL L^{-1} + interval every two weeks; E = biofertilizer 10 mL L^{-1} + interval once a week; F = biofertilizer 10 mL L^{-1} + interval every two weeks; G = biofertilizer 15 mL L+ interval once a week; H = biofertilizer 15 mL L^{-1} + interval every two weeks Data were analyzed using Analysis of Variance (ANOVA) for the F test at a 95% confidence level. Duncan's further test was carried out at a 95% confidence level if there were differences between treatments.

The first step was land preparation making a shade made of bamboo and a roof using paranet with a 50-60% density. Preparation of Class C or not ready to distribute tea seedlings were picked eight months old, 12-13 cm tall, and with less than five leaves. Seedlings were in polybags with the same topsoil and subsoil planting media 14 cm x 20 cm as the nursery. Then the treatment application, urea fertilizer, was given by dissolving it in 1 liter of water and watering the soil. The application of biological fertilizers was carried out for ten weeks, with ten applications for the once-a-week treatment and five times every two weeks.

The biofertilizer was dissolved with 1 liter of water, then watered into the soil. The maintenance included watering, weeding, and controlling pests and diseases. Watering was done every six days. Weeding was done mechanically when weeds were growing around the polybags. Pest control was carried out by spraying pesticides when the tea seedlings had been damaged by >50%. The parameters observed consisted of supporting and main observations. The main observations were made on tea seedlings' morphological and physiological conditions, including plant height was calculated using ruler, leaf area was calculated using Image J software, number of leaves, leaf chlorophyll index was calculated using Digital Chlorophyll Meter clamped on the third leaf of the sample plant until a number appears on the monitor expressed in Chlorophyll Content Index (CCI) units, and root length observation of the length of the root is done in a way dismantling sample plants. Roots were taken from the media then the measurement was made every start from the base of the stem to the longest root end. The measurement of parameters is carried out every two weeks on three plants per treatment, but the root length measurement was carried out at the end of the study. Supporting observations included soil analysis, rainfall, air temperature, humidity, and plant pests and diseases.

Result and Discussion

Soil Analysis. Based on initial soil analysis, the soil used had a pH value of 5.14. According to Thamrin *et al.* (2013), tea plants can grow optimally on land with a pH of 4.5 – 5.5. The C-Organic soil content of 8.87% is classified as very high because the Andisol order contains relatively high organic matter (Wulansari & Pranoto, 2018). The value of the cation exchange capacity (CEC) was 19 cmol $kg^{-1,}$ which was relatively high. A soil with a high CEC indicates that it is fertile because it has a high nutrient reserve (Susanto, 2005). The higher the organic matter content, the higher the CEC (Rosmarkam & Yuwono, 2002). C-Organic in the soil is useful as a source of energy for soil microorganisms, improving degraded land and increasing plant productivity (Nurida & Jubaedah, 2014).

N, P, and K Nutrients play an important role in plant growth and development. The total N content of the soil was 0.46% which was classified as moderate. P-total of 196.75 mg/100g, classified as very high, and available K-available of 18.28 mg/100g, classified as low. The available P of 0.03 ppm was very low. This is because the pH of the soil was acidic, and the soil type was Andisol. Firnia (2018) stated that P is well available in the soil at pH 6.0-7.5. Al and Fe content will be high when the pH is low and cause it to absorb very strongly but release P very slowly (Azurianti et al., 2022).

Plant height. Plant height observations were carried out 12 weeks after application (WAA). The results showed that the application of biological fertilizers did not significantly affect the height of tea seedlings at 12 WAA.

Biological fertilizers did not give a significantly different effect, probably because the biological fertilizers could not provide sufficient nutrients for the seedlings, especially P nutrients. In line with Antralina et al. (2015), microbes need a long process to adapt and grow,

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then develop and break down nutrients. Microbial life is influenced by soil organic matter, pH, temperature, aeration, and groundwater. The soil analysis results showed that the total N content of the soil was 0.46% which was classified as moderate. P-total of 196.75 mg/100g was classified as very high, and available K-available 18.28 mg/100g was classified as low. The available P of 0.03 ppm is very low.

Table 1. Effect of biofertilizer on plant height of tea seedlings 12 WAA

Treatments	Plant height (cm)
A: Control (without fertilizer)	13.09
B: Urea fertilizer every two weeks	13.70
C: Biofertilizer 5 mL L^{-1} + Interval once a week	13.53
D: Biofertilizer 5 mL L^{-1} + Interval every 2 week	14.49
E: Biofertilizer 10 mL L^{-1}+Interval once a week	13.80
F: Biofertilizer 10 mL L^{-1} + Interval every two week	14.09
G: Biofertilizer 15 mL L^{-1} + Interval once a week	14.42
H: Biofertilizer 15 mL L^{-1} +Interval every two week	14.13

Note: Numbers that are not followed by letters in the same column show no significant effect according to the F test at a 5% significance level

Furthermore, it is suspected there were indigenous microorganisms that made the plant height with and without the application of biological fertilizers not significantly different. Indigenous microorganisms are found naturally and benefit humans (Batubara et al., 2015) as decomposers of organic matter, stimulate plant growth, and control agents for plant diseases and pests (Hajama, 2014).

Leaf Area. Leaf area is one of the main observation parameters to determine the effect of biological fertilizers on leaves. The leaf area is related to plant growth because it is related to the ability of plants to photosynthesize. Based on the results, the application of biofertilizers did not significantly affect tea seedlings' leaf area (Table 2). The application of biological fertilizers was not able to increase leaf development. This was suspected due to indigenous microorganisms present in the soil. The microbial activity contained in biological, therefore fertilizers was not significant.

Table 2. Effect of biofertilizer on leaf area of tea seedlings

Treatments	Leaf area (cm^2)
A: Control (without fertilizer)	94.38
B: Urea fertilizer every two weeks	102.22
C: Biofertilizer 5 mL L^{-1} + Interval once a week	107.37
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	105.29
E: Biofertilizer 10 mL L^{-1}+Interval once a week	113.27
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	101.51
G: Biofertilizer 15 mL L^{-1} + Interval once a week	113.81
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	114.39

Note: Numbers not followed by letters in the same column show no significant effect according to the F test at a 5% significance level.

The increase in leaf area is related to nutrients such as N, P, and Mg elements. This is the opinion of Lakitan (2001), who states that the element N greatly affects the growth and development of leaves.

The increase in plant leaf area can be determined by the number of carbohydrates allocated to the leaves, so the distribution of carbohydrates into leaves greatly determines plant development. The leaf area influences planting density and supply of nitrogen nutrients (Goldsworthy and Fisher, 1992).

Number of Leaves. Based on the statistical analysis test results, the application of biological fertilizers significantly affected the number of tea seedling leaves at 12 WAA (Table 3). This is presumably due to the role of cytokinin hormones contained in biofertilizers. The analysis of biological fertilizers showed a cytokinin content of 95.60 ppm. Cytokinin hormones have an important role in regulating cell division and stimulating the growth of the number of leaves (Widiastoety, 2014). Cytokinins are circulated to the leaves from the roots through the xylem vessels and balance the protein and chlorophyll content in the leaves (Loveless, 1991).

The application of urea fertilizer and biofertilizer had different effects on controlling or without fertilizer for the number of tea seedling leaves. Urea fertilizer which contains 46% of N nutrients, is used by seeds to stimulate the growth of the number of leaves. According

to Widianto (2002), nitrogen is the nutrient that encourages leaf growth. Fauziah et al. (2015) stated that the growth of stems and leaves requires a lot of N. meanwhile, the provision of biofertilizer gave the same effect as urea giving; it showed that N content in biofertilizer gave the same effect as inorganic fertilizer, even more, significant for several treatments. This is probably supported by the function of biofertilizers that affected not only the soil's chemical properties but also improved the soil's physical character.

Table 3. Effect of biofertilizer on the number of tea seedling leaves

Treatments	Number of Leaves (strands)
A: Control (Without fertilizer)	6.00 a
B: Urea fertilizer every 2 weeks	7.00 b
C: Biofertilizer 5 mL L^{-1} + Interval once a week	7.17 b
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	6.67 b
E: Biofertilizer 10 mL L^{-1}+Interval once a week	7.92 b
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	6.83 b
G: Biofertilizer 15 mL L^{-1} + Interval once a week	7.25 b
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	8.08 b

Note: Numbers followed by the same letters in the same column show no significant effect according to the Duncan test at a 5% significance level.

The nutrients contained in the treatment can be absorbed by plants optimally. A large number of leaves made it possible to absorb more leaves so that the photosynthesis process could be faster, thus adding new leaves. On the other hand, the application without fertilizer showed a smaller number of leaves. This was because there was no addition of fertilizer for plant needs and only relied on the availability of nutrients in the soil. Hanafiah (2005) states that nitrogen fertilizer plays a prominent role in the vegetative parts of plants, namely leaves and shoots.

Chlorophyll content index. Based on the statistical analysis, the application of biological fertilizers significantly affected the chlorophyll index (Table 4).

Table 4. Effect of biofertilizer on chlorophyll content index

Treatments	Chlorophyll content index (cci)
A: Control (without fertilizer)	70.40 a
B: Urea fertilizer every 2 weeks	73.90 ab
C: Biofertilizer 5 mL L^{-1}+ Interval once a week	81.89 bc
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	80.20 abc
E: Biofertilizer 10 mL L^{-1}+Interval once a week	83.96 c
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	81.85 bc
G: Biofertilizer 15 mL L^{-1} + Interval once a week	82.94 bc
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	84.14 c

Note: Numbers followed by the same letters in the same column show no significant effect according to the Duncan test at a 5% significance level.

Treatment H (application of 15 mL L^{-1} + interval of 2 weeks) and E (application of 10 mL L^{-1} + interval of 1 week) differed significantly from other treatments. This was because the biological fertilizer contains the microbe *Azotobacter* sp. This microbe is a non-symbiotic nitrogen-fixing bacterium that can increase and improve nitrogen content (Toago et al., 2017). The results of the biofertilizer analysis showed that several bacteria helped the N fixation process, including *Azotobacter* sp (2.6×10^8 CFU/mL) and *Azospirillum* sp (1.9×10^8 CFU/mL) (Laboratory of Soil Chemistry and Plant Nutrition UNPAD, 2022) The element nitrogen stimulates growth and functions for the synthesis of amino acids and proteins in plants (Subowo et al., 2010). Leaf chlorophyll content will increase if sufficient N element is available to plants to improve the photosynthesis process and produce more assimilation (Zahrah, 2011).

Treatment A had the lowest chlorophyll index. It was possibly due to green leaves, so the leaf chlorophyll was not as much as in other treatments. This is to the research of Setiawati et al. (2016), where the older the age of the plant leaves, the greener the leaf color and the higher the chlorophyll content. Leaf chlorophyll formation depends on various factors, such as temperature, light, elements of nitrogen (N), magnesium (Mg), iron (Fe), manganese (Mn),

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copper (Cu), zinc (Zn), Sulfur (S) and oxygen. (O2) (Curtis & Clark, 1950). Therefore, treatment A was insufficient in nutrition, especially N, for not being given fertilizer.

Root length. Roots are the entrance for nutrients and water from the soil, which are very important for the physiological process of plant growth. If root function is disturbed, it will cause growth disorders in the canopy. Based on the results, the application of biological fertilizers had a significantly different effect on root length (Table 5). The roots are deficient in nutrients at low nutrient concentrations and hinder nutrient distribution. P deficiency can affect root growth. This was in line with the results of the initial soil analysis that the available P content was relatively low at 0.03 ppm, even P-total was 196.75 mg/100g, which was very high, and the available K was 18.28 mg/100g which was low. According to Herrera et al. (2015), element P is a critical nutrient that affects initiation (low Pi supply). In addition, the formation is also influenced by the level also influenced by N and Fe.

Table 5. Effect of biofertilizer on the root length

Treatments	Root length (cm)
A: Control (without fertilizer)	22.03
B: Urea fertilizer every 2 weeks	26.30
C: Biofertilizer 5 mL L^{-1} + Interval once a week	25.16
D: Biofertilizer 5 mL L^{-1} + Interval every 2 weeks	23.21
E: Biofertilizer 10 mL L^{-1}+Interval once a week	22.70
F: Biofertilizer 10 mL L^{-1} + Interval every 2 weeks	23.14
G: Biofertilizer 15 mL L^{-1} + Interval once a week	26.17
H: Biofertilizer 15 mL L^{-1} +Interval every 2 weeks	27.48

Note: Numbers that are not followed by letters in the same column show no significant effect according to the F test at a 5% significance level

Another influential factor on root length is the dense soil structure, which will inhibit the rate of deeper root penetration. Because dense soil is difficult for roots to penetrate, the root elongation area is getting shorter. Soils that have a high-density level have a low total root length. Russel (1977) *in* Rusdiana et al. (2000) argue that if the soil density increases, the macro pore space decreases, and root penetration is inhibited. According to (Nugroho, 2004), the root system will grow optimally in suitable media conditions both physically and chemically.

The root length that matches the criteria for the ready-to-distribute seedling is maximum until the plant is 1 year old. The root system is positively correlated with the resulting growth. The longer the root of a plant, the higher the ability of the plant to absorb water and nutrients so that it will produce optimal growth, such as plant height, number of stalks, and number of leaflets.

Conclusion

Treatment of biofertilizers influenced the number of leaves and chlorophyll index. The application of biofertilizer with a concentration of 15 mL L^{-1} + interval of 2 weeks had a great value on the parameters of leaf number and chlorophyll content index. It is necessary to maintain optimal maintenance to support growth toward the tea seeds ready for distribution.

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Serdani AD · Sandy YA · Li'aini AS

Identification and characterization of pathogens causing diseases on *Begonia* at Eka Karya Bali Botanic Garden

Abstract. *Begonia* is one of the world's largest genera of flowering plants that spread in various tropical and subtropical regions worldwide. However, deforestation, overexploitation, climate change, and pathogen-causing diseases have threatened the diversity of begonia. Diseases on begonia need to be handled seriously because the level of spread and damage can result in the death of the plant. Thus, this study was conducted to identify the main pathogens causing diseases in the begonia which is expected to be basic information in determining the effective control treatment. As a result, begonia collections of Eka Karya Bali Botanic Garden were mainly infected by three pathogenic fungi (*Fusarium* spp., *Oidium begoniae*, and *Botrytis cinerea*) and one bacterium (*Xanthomonas begoniae*).

Keywords: Bacterial leaf spot · *Fusarium* wilt · Gray mold disease · Powdery mildew

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Introduction

Begonia (Begoniaceae) is one of the world's largest genera of flowering plants. Begoniaceae consists of 2000 species spread in various tropical and subtropical regions worldwide (Wang et al., 2020). Begonia is an erect or creeping herbaceous plant with male and female flowers in one or different individuals, watery stems, scattered leaves, clear petioles, toothed leaf edges, oval to elongated leaf shapes, protective leaves that fall off easily, asymmetrical leaf shape, and capsule-form fruit equipped with 3 seed wings measuring 0.03-0.04 mm (Siregar, 2005; Girmansyah, 2008; 2010).

Begonia is widely used as an ornamental plant (Siregar, 2017). This is because begonia has a variety of uniqueness, including beautiful flowers with attractive colors (white, yellow, orange, pink, and red); asymmetrical leaf shapes; various leaf sizes (±10 cm); and plant heights ranging from 15-30 cm. It's average size and high adaptability make begonia easy to maintain and grow as indoor ornamental plants (Wiriadinata and Girmansyah, 2001).

Today, the decline in begonia plant diversity continues to occur. This is due to deforestation, overexploitation, and global climate change (Widjaja et al., 2014). In addition, several diseases also threaten the diversity of begonia. The level of plant resistance to pathogens infection differs from one another. Some soil or airborne diseases are known to have a very detrimental attack rate on flowering plants like begonias; for example, *Sclerotiodes* disease, *Fusarium* spp., and so forth. Diseases that infect begonia need to be handled seriously because the level of spread and damage can result in the death of the plant (Wasito and Marwoto, 2003). Thus, the present study was conducted to identify the main pathogens causing diseases in the begonia collection at Eka Karya Bali Botanic Garden (Eka Karya BBG). The results of this study are expected to be basic information in determining disease control treatments for begonia.

Materials and Methods

Observations of disease symptoms in begonia were carried out at the greenhouse of Begonia Garden, Eka Karya BBG, from April to June 2019 at an average daily temperature of 21-22°C and 80-90% relative humidity (RH). Eka Karya BBG is located in Candikuning Village, Bedugul District, Tabanan Regency, Bali, at 1250-1450 m above sea level.

Isolation of pathogenic fungi was carried out at the Applied Botany Laboratory, Eka Karya BBG, while microscopic observations of pathogens were carried out at the Genetic Conservation Laboratory, Eka Karya BBG. The results of the macroscopic and microscopic disease observations were descriptively analyzed and compared with other previous studies.

The leaves of healthy and infected begonia were observed to determine the presence of disease symptoms. The macroscopic observations were made on leaf colors and disease symptoms.

Pathogen isolation was carried out in a sterile manner in a laminar airflow cabinet. The symptomatic leaves of begonia found in the Begonia Garden were cut into squares with a dimension of 1 cm × 1 cm, dipped in 5.25% NaOCl, then rinsed three times in sterile distilled water. After that, the leaves were placed in a petri dish containing potato dextrose agar and incubated at 26°C for three days. Every single colony of fungi was taken using a loop and transferred to a new media, then incubated at 26°C to obtain pure isolate. After three days, the single colony of fungi was taken using a loop and placed on an object glass, covered with a cover glass, then microscopically observed using an Olympus CX31 microscope.

Results and Discussion

Established in 1959, Eka Karya BBG has collected thousands of plants genera, including *Begonia*. This plant family (Begoniaceae) is grown in a greenhouse that stores about 300 species of *Begonia* (Fig. 1).

The observation showed that 4 types of diseases mainly infect the Begoniaceae collection of Eka Karya BBG as described below (Table 1).

***Fusarium* wilt.** *Fusarium* is a genus of fungi that causes disease in many plants. According to Trubus (2016), one of the species that infect begonia is *F. foetens*. This fungal infection causes dull green leaves with yellow spots, pale veins, and brown vascular tissue (Rosa & Moorman, 2018). Similarly, we found that begonia infected by *Fusarium* have

dull green to yellowing leaves, pale veins, and yellow to browning spots, as shown in Fig. 2a. In severe infections, the lower leaves of some begonia plants showed a wilting symptom (Fig. 2b). This is supported by Trubus (2016) and Rosa and Moorman (2018) which stated that severe infection of *Fusarium* turn leaves into yellow color and then wilt starting from the lower leaves then spread to the top of the plant, then the roots and stems rot, and the plant dies.

Fusarium spp. forms three types of asexual spores: microconidium, macroconidium, and chlamydospores. Chlamydospores are resistant spores. Most of the isolates of *Fusarium* spp. have white, purple, or pink colonies at the center of the colony. The colony will change from white to orange in isolates that form large amounts of sporodochium (Sutejo et al., 2008). Similarly, our isolate is spherical colonial with ivory white in color.

In *Fusarium* spp., conidium is formed on monophyly, long, and unbranched conidiophores as in *F. solani, F. sacchari,* and *F. verticillodes*, or formed on branched monophyalid conidiophores as in *F. heterosporous* (Fisher et al., 1983). In contrast, our isolate showed polyphyalid conidiophore as in *Fusarium* sp. found by Ngittu et al. (2014) (Fig. 3).

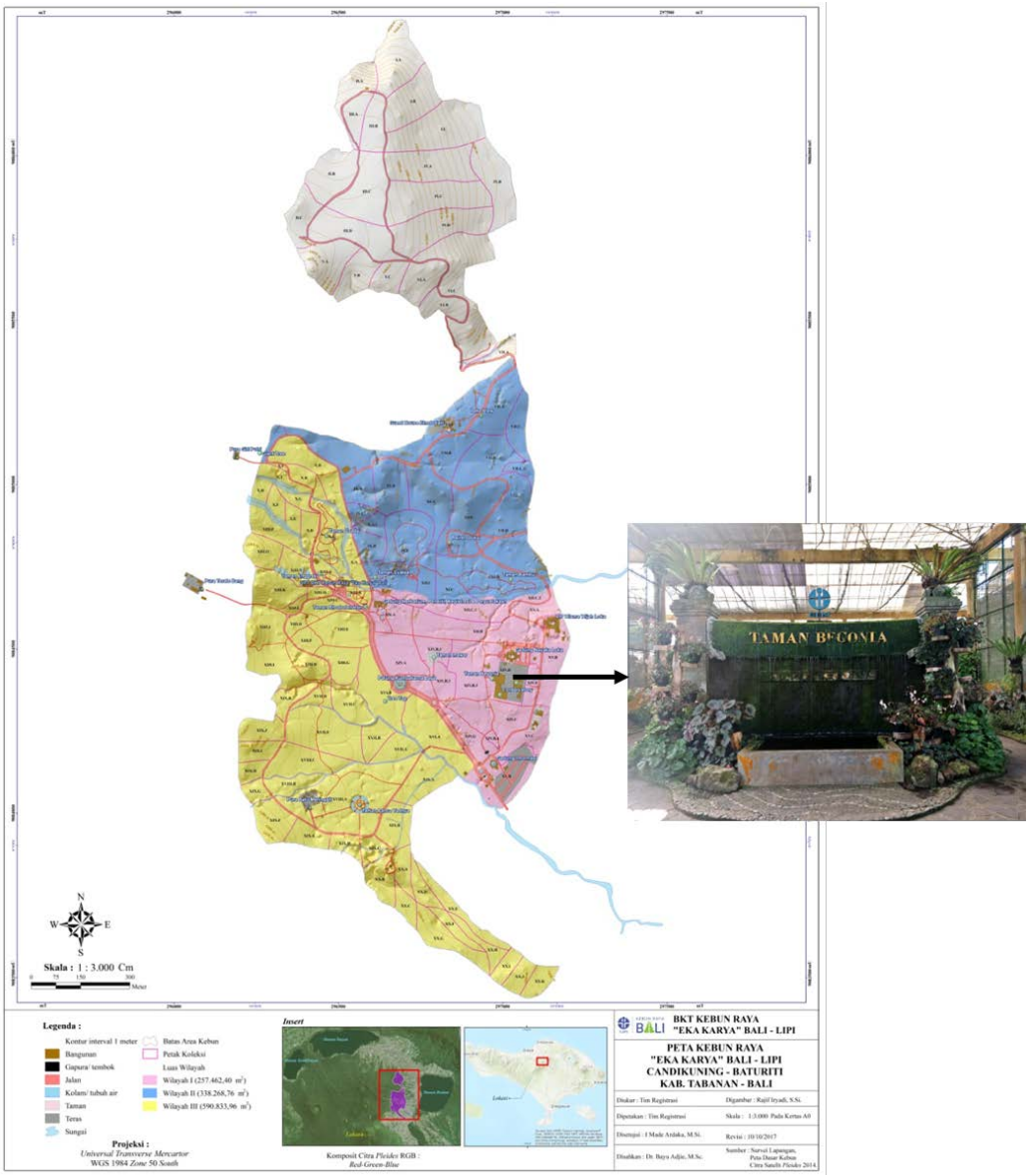

Figure 1. Location of Begoniaceae collection at Eka Karya Bali Botanic Garden (Map by the Registration Unit of Eka Karya BBG).

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Table 1. Diseases found in the begonia collection of Eka Karya Bali Botanic Garden

Plant diseases	Causal agent	Symptom
Fusarium wilt	*Fusarium* sp.	• Dull green to yellowing leaves, • Pale veins, • Yellow to browning spots, • Wilting symptoms on the lower leaves.
Powdery mildew	*Oidium begoniae*	• White or gray spots spread all over the plant including leaves, stems, and flowers, • Young shoots and leaves to curl, • Pale green leaves, necrotic, and eventually fall when the infection is severe.
Gray mold	*Botrytis cinerea*	• Pale yellow spots on the leaves, • Brown spots and blight, • Silver-gray fungal mass is seen in dead plant tissue.
Bacterial leaf spot	*Xanthomonas begoniae*	• Circular or angular yellow spots, scattered, and stiff-like blisters on the leaves, • Brown or black irregular shape (V-shaped), surrounded by transparent yellow sores.

F. foetens spores can survive in the soil for up to 30 years, infecting plants through roots or lateral growth points. The spores spread through water flow, wind, cultivation activities, and equipment. The growth of fungal spores in the vascular tissue of plants inhibits the water supply for plants, so the stomata close, and the plants die. Infection is exacerbated when excessive watering without good drainage and air circulation (Trubus, 2016).

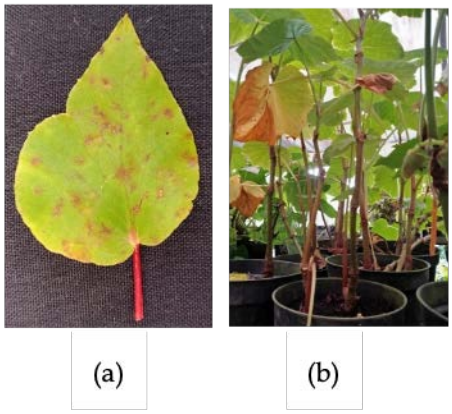

Figure 2. The infection symptoms of *Fusarium* sp. in a: *Begonia pisudoumuricata* leaf, b: *Begonia lempuyang ensis* plants in the nurseries (doc. ASL).

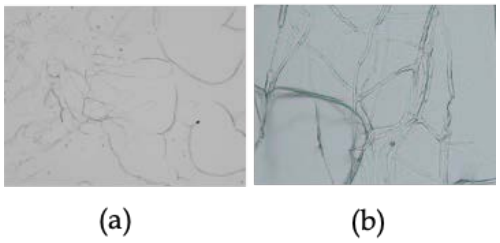

Figure 3. Microscopic cross-section of a: the chlamydospores of *Fusarium* sp. that infect *Begoniaceae* plant collections at 10x magnification (doc. ASL), b: the polyphyalid of *Fusarium foetens* that infect *Begoniaceae* plant collections at 40x magnification (doc. ASL).

Figure 4. The symptom of powdery mildew on *Begonia eka karya* plant collections (doc. ASL).

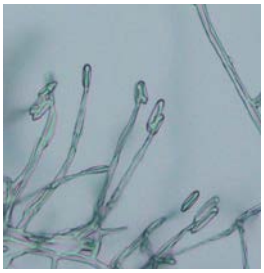

Figure 5. Microscopic cross-section of the conidiophores of *Oidium begoniae* that attack *Begoniaceae* plant collections at 40x magnification (doc. ASL).

Powdery mildew. Powdery mildew on begonia is caused by the fungus *Oidium begoniae* (Erysiphales: Erysiphaceae). *O. begoniae* is an obligate parasite (can only take nutrients from living hosts) that can cause defoliation, shoot death, and inhibit the growth of plant collections (Quinn, 1985). The infection starts from young leaves, then spreads to other parts of the plant (Kontaxis, 1985; Windham & Witte, 1998). Spores are produced in large numbers on the surface of the host so that white powder appears on the surface of the infected plant (Kontaxis, 1985; Windham & Witte, 1998). We found that powdery mildew on begonia collections of Eka Karya BBG showed a similar symptom as shown in Fig. 4. White or gray spots spread on the lower and upper surface of the leaves, stems, and flowers.

In addition, some previous studies showed that powdery mildew causes young shoots and leaves to curl, the leaves turn pale green, necrotic, and eventually fall when the infection is severe (Windham & Witte, 1998; Trubus, 2016). The infection in flowers results in deformed and low-quality flowers. Powdery mildew also prevents flowering in susceptible hosts (Windham & Witte, 1998). On older leaves with severe infection, powdery mildew symptoms are characterized by yellowish or brown spots and subsequently form necrotic spots that can reduce photosynthetic efficiency, resulting in leaf death and fall.

Similar to the previous study conducted by Putri et al. (2018) (Fig. 5a), from microscopic observations, it can be seen that the observed conidiophores of *Oidium* found from our begonia collections were elliptical and colorless (Fig. 5b). *Oidium* sp. is known as an obligate parasite that can only live on living tissue. Based on the observations, the white powdery layer is a collection of mycelium, conidium, and conidiophores of pathogenic fungi (Putri et al., 2018).

Oidium infection occurs through stomata (natural openings). Then, germinated conidia form haustoria which enter epidermal cells, and absorb nutrients contained in the epidermal cells (Boyce, 1961). Their conidia can be spread by wind, humans, cultivation equipment, or other infected plants (Kontaxis, 1985; Quinn, 1985). Furthermore, *O. begoniae* infects plants in the dry season, yet fog and high relative humidity with temperatures between 16-27°C play an important role in spore germination (Hansen, 2009).

Grey mold disease. This disease is caused by the fungus *Botrytis cinerea* (Helotiales: Sclerotiniaceae). *B. cinerea* infects plants in a cool environment with a temperature of 15°C, high relative humidity (93%), and low light, especially during the rainy season (Hausbeck & Moorman, 1996). The fungus can infect leaves, stems, crowns, buds, seeds, seedlings, tubers, and other parts of plants, except roots. Moreover, *B. cinerea* can cause the death of host cells, severe tissue damage, and decay and death of plant collections (van Kan, 2005). The conidia of *B. cinerea* can be transported by wind or water, and land on the surface of the host (Jarvis, 1977). Under optimal conditions, the disease cycle is capable of causing symptoms in only 3-4 days (van Kan, 2005).

Gray mold disease can be found in living plants with symptoms of brown spots and blight (Hausbeck & Moorman, 1996). The symptoms begin with pale yellow spots on the leaves. The spots then coalesce rapidly and widen, destroy cells and tissue, form blight symptoms (such as burning), and turn brown/black (Hausbeck & Moorman, 1996; Trubus, 2016). On the bloomed flowers, small rounded reddish-brown spots appear (Trubus, 2016). In advanced infections, plant tissue rots and dies (van Kan, 2005). These symptoms were also found in the begonia collection of Eka Karya BBG infected by *Botrytis*, as demonstrated in Fig. 6a-b. Meanwhile, in dead plant tissue, a silver-gray fungal mass is seen (Fig. 6c).

B. cinerea has hyphae shaped like bubbles bounded by white, gray, and brown partitions (Fig. 7). Then, it forms a branched and insulated mycelium. Furthermore, conidiophores appear perpendicular to the mycelium, insulated, and branched at the tips, and form a dichotomy or trichotomy. The older the conidiophores are, the

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browner they are at the tips and lighter towards the branches. The tip of conidiophores swell to form an ampulla and there is a denticle as a place for the conidium to attach (Komalaningrat et al., 2018).

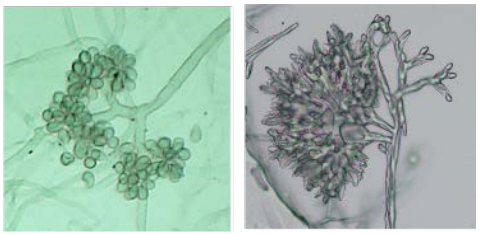
(a)

(b)

(c)

Figure 6. The symptoms of gray mold disease in *Begoniaceae* plant collections are a: brownish yellow spots on leaf surfaces of *Begonia albopicta*, b: silver fungal mycelium visible on dead leaf tissue, c: silver-grey fungal mycelium seen on a dead leaf (doc. ASL).

Figure 7. Conidia of *Botrytis cinerea* were assembled at the tip of the conidiophores at 40x magnification (doc. ASL).



(a) (b)

Figure 8. The infection symptoms of *Xanthomonas begoniae* causing bacterial leaf spot disease in *Begoniaceae* plant collections are a: the infection begins with brownish yellow spots starting from the leaf margins, b: the advanced infection with V-shaped "burns" symptom surrounded by yellow spots found on *Begonia argenteoguttata* (photo by ASL).

Bacterial leaf spot

Leaf spot disease is initiated by the bacterium *Xanthomonas begoniae* (Xanthomonadales: Xanthomonadaceae) which is carried by seeds. The further spread might be aided by splashing water, cultivation equipment, through insect's intermediary, or from the remaining infected plant collections. This disease can be very damaging to plant collections that flourish in nurseries and greenhouses with high humidity.

The bacterial infection of *X. begoniae* is indicated by circular or angular yellow spots, and scattered, and stiff-like blisters. The symptoms are first seen on the lower surface of the leaves, near the leaf margins or main vessels. The spots then coalesce, widen, and dry. The sores turn brown with an irregular shape (V-shaped in some hosts), surrounded by transparent yellow sores visible on both leaf surfaces. This pathogenic bacterium has typical symptoms: the leaves look water-soaked and withered, and there are spots of chlorosis and necrosis (Asrul et al., 2019). Similarly, the v-shaped spots found on the begonia collection were infected by *Xanthomonas* (Fig. 8). In *Xanthomonas* leaf blight, necrotic spots are brown (Schwartz & Gent, 2005). The advanced infection causes the leaves to wilt and fall.

The arrangement of plant collection at the Indonesia Botanical Garden is based on taxonomy, use, and origin (Li'aini & Kuswantoro, 2023). In this case, at Eka Karya BBG, the begonia plant collection is planted in a greenhouse. This condition is profitable for phytopathogens. Moreover, the environmental conditions of Eka Karya BBG, which have low

temperatures and high humidity, are suitable for the development of phytopathogen, especially pathogenic fungi that are mainly found to infect the begonia plant collection. The interaction between susceptible plants and virulent pathogens in a suitable environment for the growth of pathogens will cause plant diseases. Therefore, safe control techniques that can suppress the growth of pathogens are needed.

Several control techniques that can be used include sterilizing seeds (before planting) and planting equipment (Wati et al., 2021). Moreover, the planting equipment for healthy and infected plants should be separated. Another way is by eradicating or destroying diseased plants. Botanical pesticides and biocontrol agents can also be used to control plant diseases. A previous study showed that *C. aeruginosa* extract contains curcuminoids that play the role of an effective antifungi (Sari & Li'aini, 2020). Furthermore, *Bacillus amyloliquefaciens* was found to be a potential agent to control *Xanthomonas* (Li'aini et al., 2017), while Trichoderma asperellum showed an effective control against pathogenic fungi.

Conclusions

We found four microorganisms causing diseases on the begonia collection of Eka Karya BBG. There was *Fusarium* wilt, powdery mildew caused by *Oidium begoniae*, grey mold caused by *Botrytis cinerea*, and bacterial leaf spot caused by *Xanthomonas begoniae*. This information can be used to determine the effective disease control treatment in begonia collections, especially in the Indonesian Botanical Gardens.

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Fitriatin BN · Budiman MN · Suryatmana P · Kamaluddin NN · Ruswandi D

Phosphate availability, P-uptake, phosphatase, and yield of maize (*Zea mays* L.) affected by kaolin based P-solubilizer and P fertilizer in Inceptisols

Abstract. Inceptisols have problem in phosphate availability. Soil P content is very low available to plants because it is bound by soil colloids. One of the efforts to increase the P nutrient in the soil in a sustainable way by using P-Solubilizers that can dissolve phosphate in the soil so that it is available for plants. The purpose of experiment was to determine the effect of the combination dose of kaolin based P-Solubilizer and P fertilizer for improving P availability, P uptake, phosphatase, and maize yield on Inceptisols. The kaolin-based P-Solubilizer was used a consortium of phosphate solubilizing microbes (PSM) consisting of *Bacillus subtilis*, *Burkholderia cepacea, Pseudomonas mallei*, and *Trichoderma asperellum*. This experiment was conducted in the experimental field of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, from July to December 2021. The experiment used a randomized block design (RDB) method with nine treatments and three replications, with details of 0 P-Solubilizer + 0 P-fertilizer; 100% P-fertilizer; 100% P-solubilizer; and combination 50%, 75 %, 100%, and 150% P-solubilizer with 50%, 75%, and 100% P-fertilizer. P-solubilizer 100% recommended dose 50 kg ha^{-1} and P-fertilizer recommended dose 100 kg ha^{-1}. The results showed that the dose of 100% P-Solubilizer (50 kg ha^{-1}) + 75% P (75 kg ha^{-1}) showed the best results in increased P-availability (346,93%), P-uptake (312,5%), Phosphate activity (33,5%), and maize yields (48,09%) compared to without application of P-solubilizer and P-fertilizer. This consortium isolate could be developed as a P-Solubilizer with the ability to increase the efficiency of P up to 25%.

Keywords: *Burkholderia* · Efficiency · Microbes · P-solubilizing · *Trichoderma*

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Introduction

Maize (*Zea mays* L.) is the second staple commodity after rice in Indonesia. Apart from being a food commodity, maize is an important raw material for animal fodder. The need for fodder maize has not been fully met by domestic maize production, most of it comes from imports. One of the efforts that can be made to increase maize production is by extensification. In general, maize plants can grow in almost every type of soil. Inceptisols are one type of agricultural soil in Indonesia that has the widest distribution in Indonesia. However, this land has problems in its utilization, especially its low fertility rate (Muslim et al., 2020). To overcome the problem of soil fertility, biofertilizer can be applied to inceptisols to reduce the use of chemical fertilizers.

Biofertilizers are fertilizers that contain beneficial microbes that can facilitate the availability of nutrients for plants that are environmentally friendly (Kumar et al., 2022). One of the biofertilizers that can increase the availability of soil phosphate is phosphate solubilizing microbes. Phosphate solubilizing microbes (PSM) are soil microbes that can to release P from bonds with Al, Fe, Ca, and Mg, so that it can dissolve P that was originally unavailable to plants to become available to plants (Alori et al., 2017; Tian et al., 2021). This is because the PSM secrete organic acids which can form stable complexes with P-binding cations in the soil. In this study, PSM isolates were used consisting of *Bacillus subtilis, Pseudomonas mallei, Burkholderia cepacia,* and the fungus *Trichoderma asperellum.* Recently, the genus *Burkholderia* has become important as a solvent microbe (Moreno-Conn et al., 2021).

Using the PSM consortium as a biofertilizer is expected to increase the availability of soil P to be absorbed by plants to increase fertilization efficiency while reducing the use of phosphate chemical fertilizers. Sarmah and Sarma (2022) stated that the application of phosphate-dissolving microbial biofertilizers can increase nutrient availability, plant production and be able to substitute inorganic P fertilizers. This is in line with the results of research by Timofeeva et al. (2022) that PSM can increase soil available P, soil fertility, and crop production in a sustainable way.

The effectiveness of microorganisms used as phosphate-solubilizing microbes depends on environmental factors, survival in the soil, formulation quality, and applications (Raymond et al., 2021). Bacterial and fungal cells can be immobilized in solid carriers for preservation and protection from the external environment. Carriers have an important role in maintaining the effectiveness and survival of microbes during storage (Aksani et al., 2021). According to the results of a study by Herrera-Téllez et al., (2019), kaolin as a carrier was able to maintain the viability of *Trichoderma asperellum* around 1×10^7 CFU g^{-1} soil, and this level was maintained throughout the experiment (90 days), indicating the retention and survival of *T. asperellum* which is optimal in kaolin formulations.

Based on the description above, the application of kaolin-based P-solubilizer combined with P fertilizers in this study was expected to influence P availability, P uptake, phosphatase activity, and maize yields in Inceptisols.

Materials and Methods

This experiment was conducted in the experimental field of the Laboratory of Soil Chemistry and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, from July to December 2021. P-solubilizers were made at the Soil Biology Laboratory, Faculty of Agriculture, Padjadjaran University. Maize seeds used the Padjadjaran 1 variety. The PSB isolates used in this study were (*Bacillus subtilis, Burkholderia cepacia*, and *Pseudomonas mallei*) collection of Laboratory of Soil Biology Faculty of Agriculture, Universitas Padjadjaran, and the PSF isolates (*Trichoderma asperellum*) collection of Laboratory PT. Agritek Tani Indonesia. Carrier that P-solubilizer used is 100 g sterilized kaolin meanwhile inorganic fertilizer that used is Urea (46% N), SP-36 (36% P_2O_5), and KCl (50% K_2O), as well as manure from sheep manure 2 ton ha^{-1}.

The experiment used a randomized block design (RDB) consisting of nine treatments and three replications for each treatment. The treatments were: 0% P-solubilizer + 0% P (without P-solubilizer and without P-fertilizer); 100% P-fertilizer; 100% P-solubilizer; and combination 75 % and 100% P-fertilizer with 50%, 75%, and 100% P-solubilizer. P-solubilizer 100% recommended dose 50 kg ha^{-1} and P-fertilizer recommended dose 100 kg ha^{-1}.

P-Solubilizer Preparation. PSMs isolate were refreshed in agar medium (NA and PDA). Isolate propagation in NB (1% (v/v)) incubated for 3-5 days. Inoculation into the bulking media (mixture of molasses (5%) and potato extract 2:1) and incubated for 3 days. Inoculation into 100g sterile kaolin (carrier) (5% v/w), which was incubated for 5 days. PSMs population in biofertilizer counted by the Dilution Plate Count method.

Application of treatments. Two seeds of maize in a planting hole by 5 cm deep. P-solubilizer dissolved in water with a concentration of 5 $g.L^{-1}$; therefore P-solubilizer was applied directly into the planting hole with a dose according to the treatment. The treatment of inorganic P Super Phosphate 36 (P) was carried out at 1 WAP with a dose according to the treatment. Inorganic fertilizer such as Urea fertilizer at a dose of 350 kg ha^{-1} was applied 25% at 1 week after plant (WAP), 50% at 4 WAP, and 25% at 6 WAP. KCl fertilizer at a dose of 50 kg ha^{-1} applied at 75% at 1 WAP and the remaining 25% at 4 WAP recommended dose (Ministry of Agriculture, 2020). Observations were made every two weeks until 8 WAP.

Soil and Plant Sampling. P-availability, phosphatase activity, P uptake, and maize yield components were further conducted. Soil sampling was performed a week before planting and when plants reached their peak vegetative phase (56 days after planting). Analysis of the soil biological and chemical properties was taken from soil around the roots (rhizosphere).

Phosphatase enzyme activity was determined according to Eivazi and Tabatabai method. p-nitrophenyl was added to the substrate to form p-nitrophenol compound through enzyme activity. Then, it was consecutively stained by sodium hydroxide solution, which can be detected by 400 nm spectrophotometer.

P-Availability was determined according to Olsen and Bray I method. Soil samples from the field were air-dried, ground, sifted using a 2 mm sieve, and then put in a labeled plastic bag. Analysis of soil characteristics was carried out on soil properties that were thought to be closely related to soil P availability. The P content in the soil was estimated using 25% HCl extractor, Olsen, and Bray I.

Sampling for P uptake was carried out during the maximum vegetative period. The plant sample taken for analysis of plant P uptake was the 4^{th} leaf which is assumed to be an indicator leaf. The 4^{th} leaves were cleaned of adhering dirt, then air-dried, then cut into pieces, and dried using an oven at 87^0C. The dried leaves were then crushed using a grinder machine with a fineness of 0.5 mm and put into a film bottle, and labeled according to the treatment for further analysis in the laboratory. Plant P content was analyzed by the Kjeldahl method.

Harvesting was done when the cobs or husks were dry, the seeds were shiny, hard, and when pressed with a fingernail, it didn't leave an impression or at 99 days after planting (DAP). Furthermore, dried for 2 days and peeled, maize yield components as dry weight cob stated in dry weight/plant.

The data were analyzed by means of variance (ANOVA) using SPSS 25.0; for treatments that had a significant effect, Duncan's multiple distance test was carried out at a significance level of 5%.

Results and Discussions

Soil P-Availability. The results of laboratory analysis of the value of soil P availability showed in Table 1. The results of the statistical analysis showed that the effect of the application of kaolin biofertilizer combined with P fertilizer had a significant effect on the availability of P in the soil. The highest available P content was indicated by treatment 50% P-Solubilizer + 75% P. his treatment showed the highest available soil P among all treatments, namely 17.05 ppm P, an increase of 451.78% compared to the 0% P-solubilizer + 0% P, while the 0% P-solubilizer + 0% P treatment showed the lowest. This is consistent with the statements of Amri et al. (2022), P-solubilizers with consortium PSM can increase the availability of P to plants, thus promoting plant growth and making the use of inorganic fertilizers more efficient.

As shown in Table 1, overusing biofertilizers did not significantly affect the availability of P. Application of P-fertilizers only increases yield to an optimal point. It is believed that the application thickens the soil solution and prevents it from being taken up by plants. This was in line with Barłóg et al., (2022), who stated that even nutrients contained in fertilizers are not available to plants because over-fertilization leads to lower plant growth and

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enriches the soil solution. High P content in soil interferes with the uptake of other elements in the soil and hinders plant growth.

Table 1. Effect of biofertilizers on soil P–availability and P-uptake

Treatments	P availability (ppm P)	P Uptake (g plant^{-1})
0% P-solubilizer + 0% P	3.09 a	0.80 a
100% P	7.21 bc	1.43 b
100% P-Solubilizer	9.40 cd	1.36 b
100% P-Solubilizer + 50% P	14.40 e	2.82 e
100% P-Solubilizer + 75% P	13.81 e	3.30 f
100% P-Solubilizer +100%P	10.73 de	1.90 c
50% P-Solubilizer + 75% P	17.05 f	2.45 d
75% P-Solubilizer + 75% P	12.41 de	2.51 de
150% P-Solubilizer + 75% P	12.25 de	2.82 e

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

P-Uptake. Statistical test results showed that applying a combination of P-solubilizer and P fertilizer at several dosage combinations, shown in Table 1, had a significant impact on maize's ability to uptake P nutrients. The 100% P-Solubilizer + 75% P treatment increased P-uptake by 312.5% compared to 0% P-solubilizer + 0% P treatment. This is caused by P-solubilizer containing phosphate-soluble microorganisms, whose populations are fairly high at ± 10^{12} cfu g^{-1}, and can dissolve nutrient P for plant uptake. In addition to being a phosphate-soluble microorganism used in this experiment also have properties such as plant growth-promoting rhizosphere bacteria (PGPR).

PGPR stimulates root growth through the production of phytohormones (auxin, IAA), secondary metabolites, and enzymes Chandran et al. (2021). In line with the study by Shen et al. (2018), increased plant P uptake is known to be affected by P availability, root spread, and root P uptake capacity. According to Lugli et al. (2020), phosphorus uptake is highly dependent on root contact with phosphorus in dissolved soil, and the distribution of roots in soil can be very significant to increase phosphorus uptake and plant dry weight. Phosphorus uptake by maize roots is affected by the type of root and the type of soil supplied with phosphorus (Gong et al., 2022).

Activity of Phosphatase. Based on data in Table 2 showed that the application of the P-solubilizer + P fertilizer between different doses of treatment did not show a significant difference in the phosphatase activity. However, the application of a dose of 100% P-solubilizer + 75% P and 100% P-solubilizer + 100% P showed the activity of phosphate increasing. although statistically is not significant. The application of a dose 100% P-solubilizer + 75% P and 100% P-solubilizer + 100% P by increasing 33.5% and 36% compared to 0% P-solubilizer + 0% P treatment.

The increasing phosphatase activity shows the efficiency and effectiveness of nutrient uptake (Janes-Bassett et al., 2022). Without P-solubilizer the phosphatase activity tends to be lower, while in the treatment of 150% biological fertilizer + 75% P the phosphatase activity also tended to be low, this indicates that the addition of more than 100% biological fertilizer did not increase the phosphatase activity.

Fitriatin et al. (2020) revealed that PSM isolates in various rhizospheres have the ability to dissolve P by producing organic acids, phosphatase enzymes and phytohormones. Phosphatase enzymes dissolve insoluble cation-bound P complexes, making them available for plant uptake. Secretion and phosphatase activity are ways in which some microbes and plants respond to soil acidity and P deficiency (Wu et al., 2018; Nannipieri et al., 2021)

Table 2. Effect of biofertilizers on phosphate activity

Treatments	Phosphatase (μ g^{-1} h^{-1})
0% P-solubilizer + 0% P	15.11 a
100% P	18.27 ab
100% P-Solubilizer	14.81 a
100% P-Solubilizer + 50% P	18.07 ab
100% P-Solubilizer + 75% P	20.18 b
100% P-Solubilizer + 100% P	20.56 b
50% P-Solubilizer + 75% P	16.20 ab
75% P-Solubilizer + 75% P	17.54 ab
150% P-Solubilizer + 75% P	17.45 ab

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

Yields of Maize Plants. Based on statistical test showed that the application of kaolin based biofertilizer and P fertilizer had a significant effect on the dry weight of maize (Table 3). The increase of dry weight cob per hectare reached 48,09% compared to 0% P-solubilizer + 0% P treatment and 15.8% from the average dry

harvest weight of the Padjadjaran 1 variety of 11.92 ton ha^{-1}.

Table 3. Effect of biofertilizers on maize yield components

Treatment	Maize Yields Components	
	Weight of 100 Seeds (g))	Dried weight cob (ton Ha^{-1})
0% P-solubilizer + 0% P	24.20 a	8.32 a
100% P	28.61 bc	9.84 bc
100% P-Solubilizer	26.76 ab	9.21 ab
100% P-Solubilizer + 50% P	34.13 d	11.74 d
100% P-Solubilizer + 75% P	40.15 e	13.81 e
100% P-Solubilizer + 100% P	39.20 e	13.48 e
50% P-Solubilizer + 75% P	31.90 cd	10.97 c
75% P-Solubilizer + 75% P	26.53 ab	9.13 ab
150% P-Solubilizer + 75% P	31.40 cd	10.80 cd

Description: The same letter in the same column shows no significant difference according to the DMRT test with a level of 5%

Dry harvest weight maize on treatment 100% P-Solubilizer + 75% P reached 13.81 ton ha$^{-1,}$ and 100% P-Solubilizer + 100% P reached 13.48 ton ha^{-1}. From an economic point of view, providing 100% P-solubilizer + 75% P treatment has more benefits for farmers to save cost of fertilizer procurement compared to using just P-solubilizer or just P-fertilizer.

Increasing the P-availability in the soil will optimize the supply of phosphorus nutrients for plants which are useful for increasing the rate of photosynthesis and accumulation of dry matter after the flowering phase. This has a positive impact on increasing the yield of maize seeds (Zhu et al., 2012). During the generative period, especially during the seed filling phase, sufficient availability of phosphorus nutrients is required so that maximum maize yields will be obtained (Khan et al., 2014).

Conclusion

The results showed that the application of kaolin-based biofertilizer and P fertilizer (SP-36) increased the availability of P, P uptake, and components of maize yields, but had no significant effect on the soil phosphatase activity. The application of 100% P-solubilizer + 75% P gave the best yield of 13.81 tons ha^{-1} that increased up to 15.8% of the average dry harvest weight of the Padjadjaran 1 variety of 11.92 ton ha^{-1}. This consortium isolate can be developed as a P-Solubilizer with the ability to increase the efficiency of P up to 25%.

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Effect of NPK and Bacillus-coated NPK fertilizer on biomass, nutrient content in soil and nutrient uptake by lettuce

Abstract. The Inoculation of beneficial soil microbes is an effective method for lowering doses of inorganic fertilizers. This study was aimed to observe and compare the effect of doses and formulas of Bacillus-coated NPK (BCN) and conventional NPK fertilizers on biomass, major macro-nutrient in soil and their uptake by shoots of lettuce (*Lactuca sativa* L.); as well as evaluate the potency of BCN for decreasing doses of NPK fertilizers. The greenhouse experiment was set up in a randomized block design with seven treatments and five replications. The treatments included one and a half doses of recommended NPK fertilizer and two BCN fertilizer formulas; control treatment was without any fertilizer. This experiment showed that NPK fertilizer had comparable effect with BCN on growth traits; but application of NPK and coated NPK had a potency to increase the fresh weight of lettuce up to 24-45% which was in line with the increase of shoot-to-roots ratio. The potassium (K) content in soil and their uptake in lettuce shoots depend on doses and type of NPK but Nitrogen (N) and Phosphorus (P) content in soil and in shoot were not determined by treatments. The results showed that the recommended NPK dose (200 kg/ha) for lettuce can be reduced up to 50%; moreover, 50% of BCN enabled to maintain the N, P and K uptake as well as the lettuce yield.

Keywords: Bacillus · Biomass · Plant growth · Nutrient uptake

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Introduction

Currently, food crops cultivation should be supported by proper fertilization methods to maintain soil health and plant productivity. In general, farmers apply inorganic fertilizer such as NPK compound and Urea for yield increment. Nonetheless, the excessive and long-term use of inorganic fertilizers, caused N evaporation and leaching; and P adsorption by clay. Acid fertilizer can reduce the soil pH and limit the availability of P and K. Balanced fertilization with inorganic, organic and biological fertilizers has been suggested to reduce the use of inorganic fertilizers (Rahimi et al., 2019).

Nowadays, biofertilizers utilization by farmers is less intensive than inorganic fertilizers, even though microbial biofertilizers are the key to the nutrients cycle, especially Nitrogen (N) and phosphorus (P) in soil (Bhardwaj et al., 2014). Despite the positive effect of soil beneficial microbes on plant growth, few Indonesian farmers are willing to apply biofertilizers. Coating inorganic NPK fertilizer with organic carrier-based biofertilizers is suggested to intensify the utilization of beneficial microbial by farmers and hence increase the efficiency of using inorganic fertilizers. By using microbial-coated fertilizer, the farmers apply both fertilizers simultaneously, reducing the time spent during crops cultivation.

To achieve those goal, coating NPK with drought-resistance bacteria is proposed since the water content of NPK is as low as 3%. Soil microbes with these characteristics are endospore-forming Bacillus (Toyota, 2015), currently being formulated as commercial biofertilizers. In the soil, several Bacillus species have the natural ability to convert insoluble phosphate (P) into P available for root uptake (Saeid et al., 2018). The Bacillus increase plant growth by phytohormones production. *Bacillus cereus*, *B. megaterium* and *B. subtilis* produce zeatin, zeatin riboside, zeatin glycoside, isopentyl adenine, and isopentyl adenosine (Karadeniz et al., 2006). Secretion of various gibberellins and indole acetic acid by *B. methylotrophicus* KE2 has been reported (Radhakrishnan and Lee, 2016). Moreover, Bacillus tolerates biotic stresses in soil such as salinity and heavy metals (Bal et al., 2013; Syed and Chinthala, 2015) and produce volatile secondary metabolites with antimicrobial or antifungal activity

The Bacillus produce organic acid to provide phosphate (P) through phosphate solubilizing mechanisms (Saeid et al., 2018) and hence increased the yield of paddy (Fitriatin et al., 2021) as well as leafy vegetable crops. Biofertilizers consisting of *B. subtilis, B. pumilus*, and *B. amyloliquefaciens* is reported to increase the number and weight of lettuce leaves by reducing inorganic fertilizers up to 50% (Venancio et al., 2019). The *B. subtilis* 21-1 increased the yield of Chinese lettuce and decreased soft rot disease by 23.5% and 45%, respectively (Lee et al., 2014). The *B. methylotrophicus* KE2 increasing the height of lettuce plants and improving the amino acids and minerals content of lettuce leaves (Radhakrishnan and Lee, 2016).

Formulation of bacterial inoculant based on drought-resistant Bacillus is recommended for the field with limited-irrigation or in rain-feed agricultural area. The Bacillus forms the endospore in dry condition. In Indonesia, generally farmers cultivate the vegetable in such area. Coating NPK fertilizer with liquid biofertilizer of Bacillus consortium can not only intensify the use of biofertilizers by farmers, but also increase the efficiency use of NPK fertilizer and might decrease the fertilizer dose since Bacillus is able to fix the nitrogen and solubilize the phosphate. In order to develop the BCN, previous study has had two formulas of BCN. However, their effect on crops has not been yet verified. The objective of this experiment was to observed the effect of doses and formulas of bacterial-coated NPK fertilizer BCN and conventional NPK fertilizer on the availability of biomass and the content of N, P and K nutrients in the soil and canopy of lettuce (*Lactuca sativa* L.). Furthermore, this study aimed to observe the potency of BCN fertilizer in substituting some NPK inorganic fertilizers.

Materials and Methods

The Bacillus-coated NPK (BCN) fertilizer was developed by PT Pupuk Indonesia in collaboration with the Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The pot experiment was carried out in the farmers' area that was covered with ultraviolet

plastic; in Mekarwangi Village, Parongpong District, West Bandung Regency, Indonesia, at 1,260 m above sea level. The location was in a tropical mountainous area with a temperature of 17 ^{0}C–28 ^{0}C.

The pot experiment was conducted on February-April 2021 in Inceptisols with a clay texture (7% sand, 30% silt and 63% clay) and acidity of 6.9. This soil contains 1.85% organic carbon (low), 0.22% total nitrogen (moderate), C/N 8.31 (low), potential P_2O_5 38.31 mg/100 g (moderate), available P_2O_5 14.37 mg/kg (high), potential K_2O 23.57 mg/100 g (moderate). The cation exchange capacity and base saturation of the soil were 34.29 cmol/kg (high) and 49.75% (moderate) respectively. In general, the soil was moderately fertile.

Experimental Establishment. The experimental layout was a randomized block design with seven NPK fertilization treatments and five replications. The control treatment was the recommended dosage of NPK fertilizer (16:16:16) for lettuce (200 kg/ha). The seven treatments were A: Control (without NPK fertilizer), B: 1 dose of NPK fertilizer; C: ½ dose of conventional NPK; D: 1 dose of BCN-1; E: ½ dose of BCN-1; F: 1 dose of BCN-2 and G: ½ dose of BCN-2. The dose of BCN was similar to that of NPK.

Two BCN formulas used in this pot experiment were BCN-1 and BCN-2 with 0.2% and 0.4% Bacillus liquid inoculant, respectively, in the coating process. Coated fertilizer contains four Bacillus strains isolated from the rhizosphere of vegetable plants. Each Bacillus isolate was cultured in molasses-based broth for three days at room temperature; liquid inoculant contained 10^9 colony-forming units (CFU)/mL of Bacillus.

The 17-days old lettuce seedlings were grown in 5 kg potted soil in a 25 x 35 cm polybag. The soil was mixed with manure at a rate of 30 t/ha. The NPK and BCN were applied in split application at 7 and 18 days after transplanting with half dose each. The fertilizer was placed in a 2-cm deep hole at a distance of 5 cm from the stem; then covered with soil. Pesticide Chlorpyrifos was sprayed on the 10th day at a concentration of 2 mL/L with a dose of 4 L/ha. The plants were kept in plastic houses and harvested four weeks after planting (WAP).

Parameters and Statistical Analysis. Plant height and number of leaves were measured at 3 and 4 WAP while fresh and dry weight of shoot and roots were analyzed at 4 WAP. The fresh weight of shoots and roots was determined by weighing the shoots and roots at harvest while the dry weight was obtained after the plant parts were heated at 60 ˚C to a constant weight. The ratio of shoot to root were calculated based on their dry weight (Ericsson, 1995). Leaf chlorophyll content was determined from 3 fully-opened leaves nearest the growing tip using Soil Plant Analysis Development (SPAD).

The level of N, P and K in the soil as well as in lettuce shoot were measured at 4 WAP by proximate analysis according to the method of the Association of Official Agricultural Chemists (AOAC, 2012). The absorption of N, P and K in the shoot was then calculated by multiplying the nutrient content by the dry weight. The vegetative cell and spore populations of Bacillus in the rhizosphere were counted at 4 WAP by serial dilution plate method on Tryptic Soy Agar. Spores were counted after heating at 80 ^{0}C for 15 minutes. All data were analyzed by analysis of variance and continued with Duncan's Multiple Range test at the level of 5%.

Results and Discussion

Plant Growth. During the experiment, plants grew well in the polybag without any pests and diseases (Figure 1). At the transplanting time, seedlings have 5-cm height and 2 leaves; and at four weeks after planting, the shoot height were approximately 15-17 cm with about 7-9 leaves.

Figure 1. The 16-days old lettuce grown in potted soil with different dose and formula of Bacillus-coated NPK.

In current study, the plant height and number of lettuce leaves treated with conventional NPK or coated NPK were not statistically different with the control treatment

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(Figure 2). The data verified that the soil nutrients were support the vegetative growth of lettuce. Before experiment, the C/N of soil were low enough (8.31) to mobilize the N for root uptake. Moreover, the available P in soil is high and the total K was moderate. Therefore, NPK as well as BCN application in such soil might be not effective to induce plant growth. Nonetheless, long term leafy vegetable cultivation is considered to reduce the nutrient availability in soil, then the fertilizer application in appropriate dose is suggested.

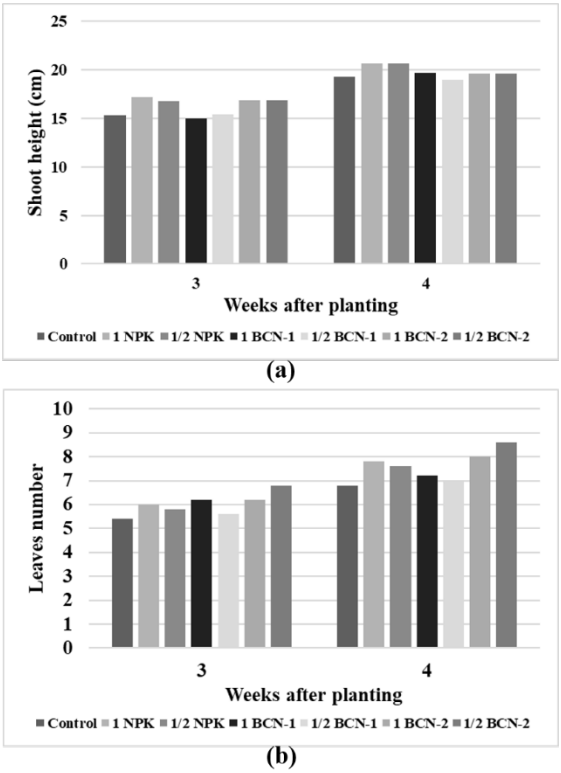

Figure 2. Shoot weight (a) and leaves number (b) of 3-weeks and 4 weeks-old lettuce after application of bacillus-coated NPK

The dose and formula of BCN also had no effect on the dry weight of roots and shoots (Table 1). All combination of NPK fertilizer dose and type caused similar growth with control (Fig 2); which is all plant are possibly produce the same quantity of photosynthate in shoot and roots. However, the application of NPK as well as coated NPK have a potency to increase the weight of shoot (edible part of lettuce) up to 24-45 % (Table 1). The used of half dose of NPK as well as BCN resulted in the same yield; which is BCN can be utilized to replace the NPK. Shoot weight with one dose of NPK and half dose of BCN was equal since the Bacillus contribute to the plant growth (Kashyap et al., 2019). Based on previous research, each Bacillus produced the phytohormones IAA, cytokinins and gibberellins which regulate plant growth and development as well as eluviate stress tolerance (Fahad et al., 2015).

Table 1. Effect of Bacillus-coated NPK on fresh and dry weight of 4-weeks old lettuce

Coated NPK treatments	Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root
Control	23.82 a	2.12 a	1.22 a	0.35 a
1 dose NPK	33.17 a	2.25 a	1.59 a	0.26 a
½ dose NPK	33.70 a	2.72 a	1.45 a	0.24 a
1 dose BCN-1	31.54 a	2.19 a	1.49 a	0.31 a
½ dose BCN-1	29.74 a	1.80 a	1.38 a	0.24 a
1 dose BCN-2	34.20 a	2.35 a	1.61 a	0.29 a
½ dose BCN-2	32.44 a	2.56 a	1.41 a	0.31 a

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 %.

Application of conventional and coated NPK clearly increased S/R (Table 2) which explained the any dose of NPK fertilizers including bacterial-coated NPK induces shoot growth. The N is a key nutrient in vegetative growth. In plant treated with BCN, shoot growth promotion was interfered by bacterial phytohormones. Auxin is important for cell elongation and cytokinin signaling involve in shoot development in tissue culture as in planta since they promote mitotic cell division of shoot (Schaller et al., 2014). Bacillus might be released available P from inorganic compound by producing organic acids (Saeid et al., 2018). The P play an important role in the synthesis of ATP for providing the energy in metabolism. This is important for tropical soils with high P adsorption capacity.

Surprisingly, plant with half dose of NPK did not show any decrease on biomass and S/R. The recommended dose of NPK is possibly too high for the leaf lettuce. However, this experiment's results align with the potential reduction of urea fertilizer by Bacillus-Azotobacter bacteria-coated urea on strawberry seedlings (Hindersah et al., 2021). Using a slightly different method, incorporating Bacillus bacteria into diammonium phosphate and Urea have a role in efficient use of the two fertilizers. in wheat plants (Ahmad et al., 2017).

Table 2 showed that variations in doses and types of BCN resulted in differences in

chlorophyll content index (CCI). Unexpectedly, the CCI of lettuce leaves without any fertilizer is comparable to that of plants with one dose of NPK; and half dose of NPK increased the CCI compared to plants with recommended dose of NPK. the application of ½ dose of BCN-1 and 1 dose of BCN-2 produced leaves with chlorophyll levels that were not different; but higher than the other treatments including control and plants with recommended dose of NPK

Table 2. Effect of Bacillus-coated NPK on shoot to root ratio (S/R) and chlorophyll content of 4-weeks old lettuce

Coated NPK treatments	S/R	Chlorophyll (CCI)*
Control	3.49 a	7.86 b
1 dose NPK	6.12 b	8.66 b
½ dose NPK	6.04 b	19.20 c
1 dose BCN-1	4.81 b	3.00 a
½ dose BCN-1	5.75 b	12.90 ab
1 dose BCN-2	5.55 b	17.22 bc
½ dose BCN-2	4.55 b	8.94 b

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 %.
* CCI: Chlorophyll Content Index

The main nutrient in chlorophyll is nitrogen. The photosynthetic process assimilating CO_2 decreases under N deficiency (Prsa et al., 2007). Bacillus has been reported to provide N through N_2 fixation (Zakry et al., 2012; Kumar, 2014). In this pot experiment, the increase in the chlorophyll content index can be attributed to the increase in the availability of N in the soil by N fixation, which enzymatically converts N_2 into ammonia. Furthermore, ammonia in the soil is reduced to NH_4^+ and through the enzymatic nitrification reaction it changes form to NO_3^- (Barth et al., 2020). Nonetheless, this current study did not analyze the available N in soil. The differences response of the plant chlorophyll index to doses and BCN formulas which might be caused by the adaptability of Bacillus in the soil, as well as differences in the effectiveness of N fixation due to different concentration of Bacillus cells on each BCN formula.

Bacillus Population in Lettuce Rhizosphere. A recommended dose of NPK, as well as full and half dose of any BCN formula resulted in more Bacillus vegetative cells in rhizosphere compared to plant without fertilizer (Figure 3).

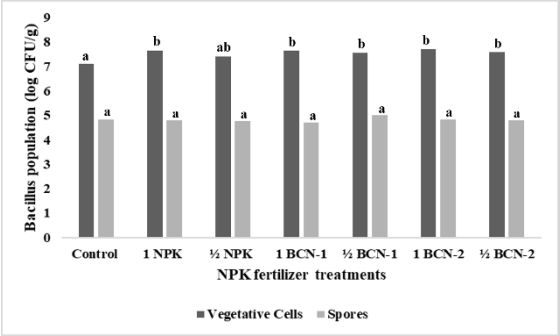

Figure 3. Population of Bacillus vegetative cells and spores in lettuce rhizosphere at 4-weeks old lettuce grown with BCN fertilizer. Different numbers on each histogram show a significant difference according to Duncan's Multiple Range test at the level of 5%

Meanwhile, the density of Bacillus spores in all treatments was not significantly different than the control. Vegetative cells were more responsive to fertilization than their spores since the NPK fertilizer is also nutrient source for bacterial cell proliferation. Sporulation from vegetative cells is particularly induced by drought environment and limited oxygen for metabolisms (Toyota, 2015). In this study, soil was always in aerobic and field capacity soil; therefore, the sporulation might not be induced.

The increase of Bacillus count in the rhizosphere might induced by IAA, Cytokinins and Gibberellins produced by Bacillus since all strains enable to synthesize these phytohormone. Auxin is a growth hormone in cell elongation as well as leaves, cambium cells and root development (Zhao et al., 2021). Gibberellins can prevent dwarf plant development (Davière and Achard, 2013) while Cytokinins increase roots cell division in the presence of an auxin (Muraro et al., 2021). In this current experiment, the Bacillus have a significant role to increase the S/R which is plant shoot (Table 1) which is stimulate growth of lettuce.

Major nutrients in soil and lettuce shoots. Duncan's test showed that the dose and type of conventional and coated NPK fertilizers did not affect the N and P content but changed the K content in soil (Table 3). At the end of the experiment, the plant soil P levels were moderate-to-high which would become

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phosphate residue for the next crop. Unexpectedly, the soil K without fertilizer was similar to the K content with full dose of conventional NPK. In general, the soil K content with the application of coated NPK at any dose was same with the soil with the control treatment.

Table 3. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium content in potted soil of 4-weeks old lettuce

Coated NPK treatments	Nutrient content (%)		
	N	P	K
Control	0.78 a	2.98 a	0.31 b
1 dose NPK	0.54 a	2.21 a	0.30 b
½ dose NPK	0.46 a	1.76 a	0.26 ab
1 dose BCN-1	0.77 a	1.43 a	0.17 a
½ dose BCN-1	0.45 a	1.86 a	0.26 ab
1 dose BCN-2	0.75 a	2.26 a	0.23 ab
½ dose BCN-2	0.65 a	1.60 a	0.24 ab

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 dose and type of NPK had no effect on N and P content and their uptake in shoots but influence K nutrients in the shoot (Table 4 and Table 5).

Table 4. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium content of 4-weeks old lettuce

Coated NPK treatments	Nutrient content (%)		
	N	P	K
Control	5.12 a	1.13 a	5.14 ab
1 dose NPK	5.48 a	1.24 a	5.19 b
½ dose NPK	5.50 a	1.18 a	5.35 b
1 dose BCN-1	5.76 a	1.35 a	4.32 a
½ dose BCN-1	4.89 a	1.20 a	5.06 ab
1 dose BCN-2	5.15 a	1.33 a	5.10 ab
½ dose BCN-2	5.61 a	1.48 a	5.13 ab

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 %.

Bacillus-based BCN had no effect on P uptake because the soil contains moderate total-N and available P. The N fixation process is inhibited by soil available N because nitrogenase which catalyzes N fixation becomes inactive (Oelze, 2000). Meanwhile the soil reaction was neutral (6.9) and the phosphate will be available for plant; in this case the Bacillus might be less involved in phosphate solubilization process. The pattern of increasing K uptake (Table 3) is in line with the soil K content (Table 4). The Bacillus probably released organic acids which were effective in dissolving K^{2+} available from inorganic K (Pramanik et al., 2019).

Table 5. Effect of Bacillus-coated NPK on nitrogen, phosphor and potassium uptake of 4-weeks old lettuce

Coated NPK treatments	Nutrient uptake (mg/plant)		
	N	P	K
Control	73.73 a	16.33 a	74.13 ab
1 dose NPK	84.12 a	17.85 a	85.21 b
½ dose NPK	79.31 a	16.99 a	77.09 ab
1 dose BCN-1	83.06 a	19.52 a	62.32 a
½ dose BCN-1	70.50 a	17.30 a	72.98 ab
1 dose BCN-2	74.16 a	19.21 a	73.46 ab
½ dose BCN-2	80.87 a	21.34 a	73.92 ab

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 %.

Current study showed that the levels of N and P in the shoots of lettuce are high but K levels are rather low. According to Jones et al. (1991), the levels of N, P and K of canopy of loose-leaves lettuce are sufficient if they contain 3.50-4.5% of N, 0.45-0.6% of P and 6.60-9.0% of K. This experiment showed that the application of recommended-dose NPK (200 kg/ha) and bacteria-coated NPK with the same dose did not increase N, P and K uptake compared to half the recommended dose (100 kg/ha).

Conclusion

The dose and formula of Bacillus-coated NPK (BCN) did not affect the height and number of lettuce leaves, biomass, soil N and P content, and N and P uptake of lettuce shoots compared to control and conventional NPK. Both NPK and BCN increased the shoot to root ratio; while only full dose of NPK have increased soil K and K uptake. Any dose and formula of BCN slightly reduce the K in soil and plant compared to NPK treatment. In current study, the population of either vegetative cell or endospore of Bacillus remain similar irrespective of NPK fertilization.

The shoot height and fresh weight of lettuce did not influence by dose and type of NPK which is verified that half dose of any NPK fertilizer produce the comparable yield, Nonetheless, the lettuce has high N and P

content but low K. The results explained that recommended dose of NPK can be reduced up to 50% by using conventional NPK or Bacillus-coated NPK. In order to increase K content in plant shoots, additional potassium fertilizer is needed. This experiment showed that conventional or microbial-coated NPK fertilizer can be applied at half dose in Andisol with moderate available N and P, high total P and moderate total K.

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Soil nutrient and invertase-producing bacteria relation impact on cilembu sweet potato (*Ipomoea batatas* L.) growth: A study based on dry fields and paddy fields cultivation in Cilembu village Sumedang district

Abstract. Environment is one important factors that must be considered in supporting sweet potato productivity. Environmental factors can be biotic and abiotic, including the availability of nutrients and microbes in the soil. This study aimed to identify the nutrient content of the soil in paddy fields and dry fields, identify the total invertase microorganisms in paddy fields and dry fields, and identify the relationship between soil nutrients and microorganisms during the growth of Cilembu sweet potato in dry and paddy fields agroecosystems. This research was conducted on dry fields and paddy fields in Cilembu Village, Sumedang Regency. The experimental design used was a randomized block design (RBD) with six treatments and three replications: A; Rancing, paddy fields, B; Biang, paddy fields, C; Mencrang, paddy fields, D; Rancing, dry fields, E; Biang, dry fields, F; Mencrang, dry fields. The observed parameters included pH, C-organic, total-N, available-P, exchangeable-K, exchangeable-Na, exchangeable-Ca, and exchangeable-Mg, cation exchange capacity (CEC), and total invertase-producing bacteria. The results showed that in paddy fields, the pH was slightly acidic, and the nutrient content such as N, available P, exchangeable Ca, Mg, Na, CEC, and C-organic tended to be more available. Soil K and abundant invertase bacteria were more available in the dry fields. The activity of invertase bacteria had a close relationship with the K content. The information generated in this study could be used to determine an effective location to produce good quality sweet potato.

Keywords: Dry fields agroecosystem · Paddy fields · Plant nutrition · Invertase-producing microbes

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Introduction

Sweet potato is a versatile food crop highly adaptable to changing environments. According to several studies, it can grow and produce optimal yields in various environments (Mustamu et al., 2018; Karuniawan et al., 2021a; Maulana et al., 2022). In addition, this plant can also be used for food, feed, and industrial raw materials (Karuniawan et al., 2021b). These numerous benefits have made sweet potato one of the leading commodities in Indonesia, particularly in West Java.

The Cilembu sweet potato is a particularly popular variety in Indonesia and the world. This type of sweet potato has its own unique advantages, including the production of honey/caramel when baked or roasted (Astawan and Widowati, 2011; Lai et al., 2013). According to Solihin et al. (2018), the quality of the honey does not show significant differences when grown in different environments. However, Karuniawan et al. (2021a) reported different results, showing that the level of sweetness is greatly influenced by the interaction between the genotype and the environment, meaning that each gene will have different potential for sweetness when grown in different environmental conditions. Currently Cilembu farmers believe planting sweet potatoes in paddy fields is more profitable than dry fields. Hence, further research is needed to better understand the level of sweetness in Cilembu sweet potato.

The land in Cilembu Village is primarily comprised of dry fields and rainfed paddy fields. Sweet potato is typically planted on dry fields at the beginning or middle of the rainy season and in rainfed paddy fields areas after the rice harvest. Solihin et al. (2017) reported that the Rancing variety of Cilembu sweet potato showed no significant differences in sweetness quality when planted in paddy fields and dry fields. However, this needs to be re-examined as sweetness quality is a quantitative value strongly influenced by the environment and can change due to the interaction of genetics and the environment.

Soil characteristics, such as nutrient availability and microorganisms, can affect plant growth and yields. Plants need both macro and micro-nutrients throughout their life cycle, and soil microorganisms play a role in breaking down organic matter and increasing nutrients (Ortiz and Sansinenea, 2022). Element K plays a crucial role in tuber production and sugar content (Anda et al., 2018). A study also found that the population of bacteria in the soil during the growth of sweet potatoes in the Cilembu area was higher compared to outside of the Cilembu area (Tangapo et al., 2018). These results indicate that nutrients and microorganisms play a role in increasing the production and quality of sweet potatoes in the Cilembu area. Therefore, this study aimed to determine the soil nutritional content and total invertase microorganisms during the growth of Cilembu sweet potato on dry fields and paddy fields in the Cilembu area and their relationship.

Materials and Methods

The methodology of the research involved selecting dry fields and paddy fields in Cilembu Village, Pamulihan District, Sumedang Regency, West Java for the study(latitude 6^{0}54′17,2′′S, longitude 107^{0}50′39,7′′E and latitude 6^{0}54′13,1′′S, longitude 107^{0}50′41,7′′E). The experiment was conducted from September 2022 to January 2023 used Randomized Block Design (RBD) with six treatments and three replications. The treatments were labeled as A (Rancing, paddy fields), B (Biang, paddy fields), C (Mencrang, paddy fields), D (Rancing, dry fields), E (Biang, dry fields), and F (Mencrang, dry fields). The parameters observed included pH (measured through electrometric method), C-organic (using the Walkley and Black method), N-total (using the Kjeldahl method), available P (using the Bray/Olsen method with spectrophotometry), base exchangeable K, Na, Ca, and Mg (measured through the NH_4OAc Extraction method and pH 7 Flame photometer measurement), Cation Exchange Capacity (measured through the NH_4OAc Extraction method at pH 7),(Soil Research Institute, 2009) and total bacterial invertase (measured through the Total Plate Count method)(Lase et al., 2021).

The experiment included several stages, including land preparation, fertilization, planting, maintenance, and soil sampling for chemical and biological analysis. The land preparation stage involved clearing the land of weeds and then loosening it to a depth of over 20 cm using a hoe. Bunds were also created, with a length of 5 m each, width of 70 cm, and a spacing of 30 cm between them. The experiment

involved planting one cutting of each genotype with a spacing of 25 x 100 cm, and fertilizing the soil with organic and inorganic fertilizers. The organic fertilizer was applied at a dose of 10 tons/ha during land preparation, while the inorganic fertilizer was Phonska NPK (16-16-16) with a dose of 200 Kg/ha, applied 7 days after planting 1/3 dose and 45 days after planting 2/3 dose.

Planting involved inserting one cutting in each planting hole with a spacing of 25 cm x 100 cm and positioning each cutting in an "L" shape. The maintenance involved watering the plants and weeding the surrounding area. Watering was done at the start of planting. The soil or mounds were dry, and there was no rain while weeding was done when the area had an excessive growth of weeds, i.e., a month after planting. Soil sampling was conducted during the final vegetative stages period, or ±2 months after planting (Anda et al., 2018), and involved taking soil samples from five points diagonally at a depth of 10-30 cm. The soil samples were mixed evenly and a kilogram was taken for testing in the laboratory to measure the chemical properties and soil biology around the plant rhizosphere.

The data from the chemical and biological properties of the soil was then analyzed statistically using IBM SPSS Statistics version 25. If the results of the analysis of variance showed a significant difference, the Duncan mean value difference test was performed at a significance level of $\alpha = 5\%$. To determine the relationship between chemical properties and soil biology, a Principal Component Analysis (PCA) was performed. The R program software was used to assist with data processing for the PCA (Jolliffe IT, 2002).

Results and Discussion

Comparison of Soil Nutrient Conditions in paddy fields and dry fields in Cilembu Village. The results of the analysis of the chemical properties of the soil in paddy fields and dry fields planted with sweet potatoes (Rancing, Biang and Mencrang) were presented in Table 1. The soil pH values in the paddy fields were found to range from 6.13 to 6.21, while in the dry fields, the pH values were from 5.86 to 5.94. According to the Soil Research Institute (2009), these values are considered to be sughtly acide. The pH condition of the soil affects the ease with which nutrient ions can be absorbed by plants A neutral soil pH is the most optimal for providing nutrients to plants as most nutrients dissolve easily in these conditions and are easily absorbed (Karamina et al., 2018).

The results of the statistical analysis for C-organic content in paddy fields and dry fields showed significant differences. The C-organic content in paddy fields (Rancing, Biang, and Mencrang planting areas) was 2.54%, 2.34%, and 2.68%, respectively, while in the dry fields it was 2.01%, 1.77%, and 1.75%. C-organic is a major constituent of organic matter and thus, an overview of soil organic matter can be obtained from its C-organic content. Organic matter can increase ability to hold and store water, besides that it is closely related to the availability of nutrients in the soil.. Nutrients are absorbed by plants in the form of ionic cations and anions, with cations being absorbed more readily (Romadhon and Hermiyanto, 2021).

The results of the statistical analysis for soil nitrogen content in paddy fields and dry fields showed significant differences. The soil nitrogen values in paddy fields ranged from 0.24% to 0.26%, while in dry fields, it was from 0.18% to 0.19%. Nitrogen is one of the essential macronutrients for vegetative processes, and its absence during growth can result in stunted plant development (Solihin et al., 2019). Nitrogen is absorbed by plants from the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-) ions (Amir et al., 2014). These forms of nitrogen have mobile properties in the soil, making them susceptible to loss through leaching, volatilization into the atmosphere, or uptake by soil micro- and macro-organisms (Kusumandaru et al., 2015).

Available phosphorus (P-available) is a phosphorus nutrient that is readily utilized by plants. Phosphorus is absorbed by plants in the form of primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) (Umaternate et al., 2014; Firnia, 2018). It is one of the essential macro-nutrients for plants, and plays a role in photosynthesis, respiration, energy transfer and storage, cell division, and enlargement (Hasibuan et al., 2014). The available P-nutrients showed significant differences between the paddy fields and dry fields (Table 1). The phosphorus content in paddy fields (Rancing, Biang, and Mencrang) was 55.32 ppm, 59.77 ppm, and 56.85 ppm, respectively, while in the

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dry fields it was 50.52 ppm, 46.23 ppm, and 48.12 ppm. The level of available P is influenced by P input and the presence of phosphate rock in the soil (La Habi et al., 2018; Nugroho et al., 2020). Continuous and excessive phosphorus (P) input leads to an increase in P levels, resulting in a surplus of P in the field (Palembang et al., 2013; Fauzan et al., 2021). Although most soils contain substantial reserves of inorganic P, most of it is in a form that is not readily accessible to plants and is absorbed in an insoluble and tightly bound state. P-organic, however, can be converted into available inorganic P through hydrolysis or mineralization processes (Spain et al., 2018).

The Cation Exchange Capacity (CEC) is a measure of the total number of cations that can be exchanged on the surface of a negatively charged colloid, and is influenced by the presence of clay and organic matter fractions in the soil (Jayanti and Mowidu, 2015). The CEC of clays refers to the total number of cations adsorbed specifically by the clay fraction. A statistical analysis of the CEC values in paddy fields and dry fields revealed significant differences. The CEC values in paddy fields planted with Rancing, Biang, and Mencrang were 21.85, 21.15, and 21.32 cmol kg-1, respectively, while the values for dry fields with Rancing, Biang, and Mencrang plantings were 16.51, 17.32, and 16.45 cmol kg-1, respectively. Soils with high CEC values indicate that they have the ability to provide nutrients in the form of exchangeable cations. CEC values are influenced by various factors, such as soil pH, texture, and organic matter content (Zgorelec et al., 2019).

Table 1 also showed that the basic cation content, including Calcium (Ca), Magnesium (Mg), Potassium (K), and Exchangeable Sodium (Na), in paddy fields and dry fields planted with three sweet potato clones showed significant differences. In paddy fields planted with Rancing varieties, the average values of basic cations, such as exchangeable Ca, Mg, K, and Na, were 9.64 cmol kg^{-1}, 3.61 cmol kg^{-1}, 0.91 cmol kg^{-1}, and 0.31 cmol kg^{-1}, respectively. For paddy fields planted with Biang, the values were 9.74 cmol kg^{-1}, 3.87 cmol kg^{-1}, 0.90 cmol kg^{-1}, and 0.38 cmol kg^{-1}, respectively. Meanwhile, those planted with Mencrang had values of 10.92 cmol kg^{-1}, 3.44 cmol kg^{-1}, 1.01 cmol kg^{-1}, and 0.33 cmol kg^{-1}, respectively. The content of exchangeable Ca, Mg, K, and Na in dry fields planted with Rancing varieties were 8.28 cmol kg^{-1}, 2.14 cmol kg^{-1}, 1.22 cmol kg^{-1}, and 0.20 cmol kg^{-1}, respectively. For dry fields planted with Biang, the values were 7.46 cmol kg^{-1}, 1.96 cmol kg^{-1}, 1.17 cmol kg^{-1}, and 0.26 cmol kg^{-1}, respectively. Meanwhile, the dry fields planted with mencrang had values of 7.64 cmol kg^{-1}, 2.37 cmol kg^{-1}, 1.27 cmol kg^{-1}, and 0.23 cmol kg^{-1}.

Ca, Mg, and K are macronutrients that are needed by plants in relatively large quantities. Mg is absorbed by plants as Mg^{2+} ions, while Ca is absorbed as Ca^{2+} ions with the same valence (Soewandita, 2008). Mg is one of the elements of chlorophyll and is involved in photosynthesis, while Ca plays a role in stimulating the formation of root hairs, hardening stems, and stimulating seed formation. If the soil is low in calcium, the leaves can easily experience chlorosis. K is absorbed by plants as K+ ions and plays a role in the efficiency of water use, such as the process of opening and closing leaf pores and stomata (Apriliani et al., 2016). It also has a role in regulatory mechanisms, such as in the process of photosynthesis, carbohydrate translocation,

Table 1. Results of soil chemical analysis in paddy fields and dry fields

Treatments	paddy fields			dry fields		
	Rancing	Biang	Mencrang	Rancing	Biang	Mencrang
pH	6.21 b	6.13 b	6.17 b	5.94 a	5.86 a	5.90 a
C-Org(%)	2.54 c	2.34 bc	2.68 c	2.01 ab	1.77 a	1.75 a
Tot-N (%)	0,26 c	0.24 bc	0.26 c	0.18 a	0.19 ab	0.19 ab
Av-P(ppm)	55.32 abc	59.77 c	56.85 bc	50.52 abc	46.23 a	48.12 ab
CEC(cmol kg^{-1})	21.85 b	21.15 b	21.32 b	16.51 a	17.32 a	16.45 a
Exch-Ca(cmol kg^{-1})	9.64 bc	9.74 bc	10.92 c	8.28 ab	7.46 a	7.64 a
Exch-Mg(cmol kg^{-1})	3.61 b	3.87 b	3.44 b	2.14 a	1.96 a	2.37 a
Exch-K(cmol kg^{-1})	0.91 ab	0.90 a	1.01 abc	1.22 c	1.17 bc	1.27 c
Exch-Na(cmol kg^{-1})	0.31 d	0.38 f	0.33 e	0.20 a	0.26 c	0.23 b

Note: The mean value followed by the same letter is not significantly different based on Duncan's multiple range test at the 5% level

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and protein synthesis. However, because K has a relatively large hydrated form and a valency of 1, it is not strongly adsorbed by soil colloidal loads, so it is easily leached. Although sodium is not an essential nutrient, its presence in the soil can sometimes replace potassium for certain plants and is known as a functional element. It can also increase the solubility of K from minerals to soil solution (Mengel and Kirkby, 2001). The presence of Na affects not only the chemical properties of the soil but also its physical properties, particularly its structural stability. Its high concentration in the soil can cause disturbances in plant metabolism and affects the osmotic properties and stability of aggregates, besides having physiological effects.

Total population of invertase bacteria in paddy fields and dry fields in Cilembu village. In this study, the average abundance of invertase-producing bacteria was observed in dry fields and paddy fields in Cilembu Village planted with 3 different sweet potato clones (Figure 1). The average yield of the abundance of invertase-producing bacteria in paddy fields planted with Rancing, Mencrang, and Biang was 2.80 x 10^5 cfu g^{-1}, 2.42 x 10^5 cfu g^{-1} , and 2.94 x 10^5 cfu g^{-1} . As for dry fields, it was 3.26 x 10^5 cfu g^{-1}, 2.75 x 10^5 cfu g^{-1} and 3.08 x 10^5 cfu g^{-1}. The average value of the abundance of invertase-producing bacteria in dry fields and paddy fields is statistically different. In this study, it was shown that the treatment of paddy fields planted with Mencrang was lower while the dry fields with sharp sedges had the highest average value compared to other treatments.

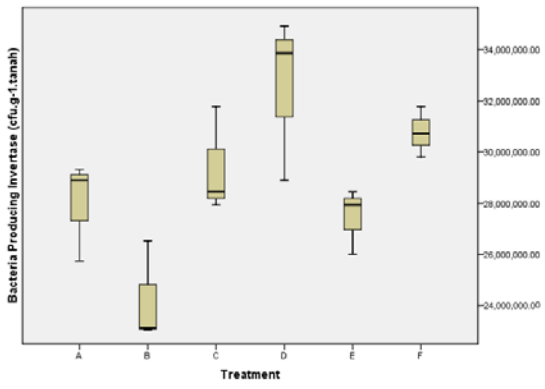

Figure 1. Box plot of the abundance of invertase-producing bacteria in the six treatments. X-axis code see material and methods section.

The abundance of invertase-producing bacteria in paddy fields and dry fields planted with three different sweet potato clones was influenced by various environmental factors, both biotic and abiotic. The content of organic matter in the environment can affect microbial populations, and in this study, it was observed that the organic matter content in dry fields was lower compared to that in paddy fields. Bacteria, as a type of microbe, play a significant role in the decomposition of organic matter through an enzymatic process. One of the enzymes produced by microbes during this process is invertase. High levels of microbial activity can affect the degradation process of high organic matter. In addition to organic matter, root exudates secreted by plants can also trigger the desired microbial growth and aggregation in the root area or rhizosphere zone. The effect of root exudation on the abundance and diversity of microbes in the rhizosphere zone is relatively higher compared to the non-rhizosphere zone (Prayudyaningsih, 2015; Nazir et al., 2016).

The Relationship between Soil Nutrients and Invertase Bacteria using Principal Component Analysis (PCA). A Principal Component Analysis (PCA) was performed to investigate the relationship between environmental factors such as the agroecosystems of dry fields and paddy fields and the total microorganisms (invertase-producing bacteria) and soil nutrition (soil chemical properties: pH, organic-C, total-N, available-P, CEC, exchangeable Ca, Mg, K and Na. The results of the PCA for the six treatments tested based on soil characteristics showed two axes with eigenvalues between 8.487 and 1.092, and a cumulative value of 95.792% (Table 2). The first component (PC1) had a variation contribution of 84.871%, where all the elements tested had a significant impact on diversity.

The second component (PC2) had a variation contribution of 10.921% and was influenced by invertase-producing bacteria. According to the data obtained, the elements tested contribute to diversity, with some elements contributing positively and others negatively (Table 2). According to Haydar et al. (2007), elements that contribute positively indicate optimal contribution, while elements that contribute negatively indicate suboptimal contribution to diversity. In PC1, pH, C, CEC, N, P, Ca, Mg, and Na contribute the most, while elements K and invertase-producing bacteria make a suboptimal contribution. However,

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invertase-producing bacteria provide the maximum contribution to PC2.

In a separate study, Markos et al. (2022) used PCA to identify the environmental contribution to the yield and quality of maize. The results of the PCA analysis showed that the properties tested had a strong relationship, indicating that soil nutrients with positive and dominant values are closely related to invertase bacteria, which will ultimately affect the quality of sweet potato yields.

Table 2. The result PCA for total microbes and soil nutrients

Componen (PC)	1	2
Acidity (pH)	**0.962**	0.232
Carbon Organik (C-or)	**0.924**	0.360
Cation Exchange Capacity (CEC)	**0.987**	-0.001
Nitrogen (N)	**0.964**	0.121
Phosphorus (P)	**0.939**	0.047
Calcium (Ca)	**0.917**	0.328
Magnesium (Mg)	**0.974**	-0.033
Potassium (K)	**-0.961**	0.175
Sodium (Na)	**0.914**	-0.364
Invertase producing bacteria	**-0.613**	**0.787**
Variation (%)	84.871	10.921
Cumulative (%)	84.871	95.792

Note: *numbers in bold indicate discriminant >0.5 or <-0.5 and contribute to diversity (Jolliffe, 2002)

The relationship between each element and treatment can be visualized from the PCA biplot graph in Figure 2. The results reveal the formation of four quadrants, including quadrants I, II, III, and IV. Treatments or elements in the same quadrant exhibit a close relationship, while those in different quadrants display no close relationship (Maulana et al., 2018). Figure 2 illustrates that the dominant elements in quadrant 1 are C, Ca, pH, N, and P. These five elements have a strong interdependence, as evidenced by the angles formed by each element. In a separate study, Aziza et al. (2021) reported that properties with an acute angle (<90˚) have a very strong relationship. Additionally, there is also a treatment in quadrant 1, namely the paddy fields planted with Rancing and Mencrang, which demonstrates that these elements in this quadrant significantly influence the diversity in paddy fields. In quadrant 2, two elements (bacteria and K) form an obtuse angle (>90˚) away from the other elements, indicating an opposing relationship with other properties. There are also two treatments in quadrant 2 that display a fairly strong relationship with these two elements, namely the upland planted with Rancing and Mencrang. In quadrant 3, there is one treatment (E), the paddy fields planted with starter, but there are no elements tested. This indicates that the treatment tends to have a negative correlation with all other elements and treatments in the test. In quadrant 4, there are three elements, CEC, Mg, and Na, and one treatment, the paddy fields planted with Biang. These three elements have an acute angle with the elements in quadrant 1, indicating a strong relationship, but they primarily influence the location of paddy fields planted with Biang.

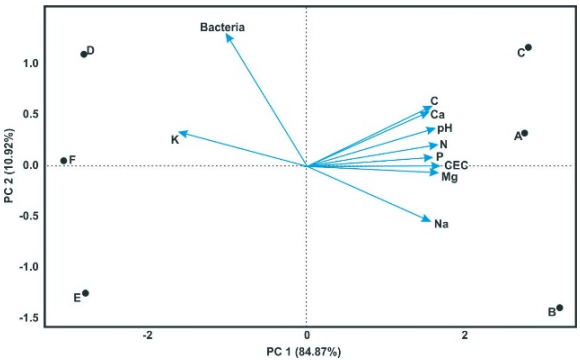

Figure 2. PCA biplot between treatments and elements tested in paddy fields and dry fields agro-ecosystems. Code see material and methods section.

The location of paddy fields has a higher availability of nutrients, such as pH, C-organic, N, P, CEC, Ca, Mg, and Na. Meanwhile, dry fields locations have more abundant nutrients for K and a greater abundance of invertase-producing bacteria. This is because paddy field has a higher organic matter content, resulting in better soil fertility. According to Mukherjee and Lal (2014), soil quality is influenced by the type of soil and its management. Improper management can cause soil damage and make it unable to support crop production. Intensive tillage in dry fields areas can reduce the content of organic matter and lead to soil acidification (Neina, 2019; Dewi et al., 2020).

Conclusion

Two agro-ecosystems have different fertility levels, both in terms of nutrition and

microbial availability, which were part of the environmental components that supported plant productivity. In paddy fields, the availability of nutrients such as nitrogen, phosphorus, calcium, magnesium, sodium, cation exchange capacity, and organic carbon were higher, while potassium content and abundance of invertase bacteria were higher in dry fields. The close relationship between the abundance of invertase bacteria and potassium content was also noted. Bacteria and potassium had implications for increasing yields and sweetness levels of sweet potato.

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In-situ characterization of Jatigede local roid banana (*Musa* spp.) based on morphological and agronomical characteristics

Abstract. Banana (*Musa* spp.) is one of the agricultural commodities that's widely cultivated and used by the local community. Currently, only 101 types of local varieties of bananas are registered in Indonesia; one of them is the Roid banana from Jatigede District. Roid bananas grow wild without any mandatory special care. Continuous use without any conservation and preservation efforts can lead to scarcity of Roid banana germplasm as a genetic resource. Therefore, it is necessary to identify the distribution and diversity of Roid bananas through *in situ* exploration and characterization activities. This study aimed to identify the potential for genetic diversity and develop conservation efforts for the local variety of Roid banana (*Musa* spp.) in Jatigede District. This research was conducted from November 2021 - March 2022 in three villages in Jatigede District: Ciranggem Village, Jemah Village, and Mekarasih Village. The research used exploratory, survey, and interview methods as data collection techniques. Based on the results of the study, Jatigede District has a wide genetic diversity and distant kinship among Roid banana accessions. Characters that contributed to genetic diversity among accessions were the width of the midrib margin, the diameter of the bunch, and the length of the pseudostem. MS1.3 was the selected accession with its potential characteristics: more combs in bunches, leaf width of 71-80 cm, leaf length of 171-220 cm, and the number of rhizomes > 5 tillers.

Keywords: Exploration · Characterization · Germplasm conservation · Index cultural significance · Principal component analysis

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Introduction

Banana is one of the agricultural commodities widely cultivated and used by people in various circles. Bananas are a fruit crop with a high level of consumption, reaching 7.2 kg/cap/year in Indonesia. Bananas are rich in vitamins, minerals, and carbohydrates; hence, they are recommended for consumption during the COVID-19 pandemic (Marpaung and Handayani, 2020). Other than directly consumed, people often process bananas into various products such as "sale" and banana flour (Putri et al., 2015). Other parts of banana plants, such as leaves, tubers, midribs, flowers, and roots, are also utilized to benefit the community. Banana leaves can be used as food wrappers, while the cob can be processed into crackers.

Banana plants in Indonesia have a relatively high level of diversity. Currently, 101 types of banana plants have been registered as local varieties in Indonesia (PPVT-PP, 2021). This number is expected to continue to increase, considering that many local varieties of bananas have not been identified. Various types of bananas are scattered in various regions in Indonesia, one of which is in West Java Province. Some types of bananas that are recorded as local varieties in West Java include Roid Jatigede bananas, Manggala Hitam Karyamukti bananas, Kole Karyamukti bananas, and Ranggap bananas (PPVT-PP, 2021).

Roid banana is a local variety in West Java that has the potential to be developed. According to the PVTPP (2017), Roid bananas have advantages in terms of fruit storage, low fruit loss rates, and resistance to pest and disease attacks. The shelf life of Roid bananas reaches 2-3 weeks or 4-11 days longer than Kepok bananas (Ikhsan et al., 2014). These bananas are found only with wild growing in Jatigede District without any treatment from the local community. Most people use Roid bananas as a source of food and household income (Masriah et al., 2019). Continuous use and lack of efforts to preserve Roid banana can trigger a scarcity of Roid banana germplasm. Therefore, conservation and germplasm management efforts are needed to preserve and develop the potential of Roid banana.

Roid banana conservation and preservation activities can optimize the use of germplasm in West Java. The purpose of germplasm is as a genetic resource that has use and economic value, as well as a means to trace the origin and authenticity of species (Sumarno and Zuraida, 2008). Conservation and preservation activities can minimize genetic erosion or species extinction rate (Pusponegoro et al., 2018). Conservation activities can be carried out in their natural habitat (*in situ*) and outside their natural habitat (*ex situ*). Research related to the Roid banana in Jatigede district has not been carried out widely , hence, any information that be obtained from the results of this study can be used to support the success of conservation efforts for Roid bananas.

Distribution identification and Roid banana's diversity are the initial actions in developing a strategy for conserving and preserving Roid bananas. These efforts can be carried out through *in situ* exploration and characterization activities. Exploration activities aimed to collect the best accessions as genetic resources to assemble new superior varieties (VUB) (Maskrono et al., 2017). Roid bananas were identified and *in situ* characterized based on their morphological and agronomical appearance. Indigenous knowledge is also needed to support the exploration and *in situ* conservation of Roid bananas. Indigenous knowledge was obtained from the questionnaires and interviews regarding the character and importance of Roid bananas for the local community. The results of this activity will show the kinship between accessions and the potential for Roid bananas in several locations (Lesta et al., 2018). In addition, indigenous knowledge also provides an overview of *in situ* conservation techniques that are appropriate to the local culture.

This research is a follow-up of the registration of local plant varieties. The existence and potential of Roid bananas, which have not been widely published, provide a great opportunity for distribution mapping of superior accessions and analysis of conservation strategies for Roid bananas in Jatigede. The potential of produced Roid bananas would be in line with the quality of the identified accessions. The information collected is useful for completing a comprehensive data collection on diversity of banana germplasm in West Java. The complete data is expected to support engineering and plant breeding activities to improve the quality of bananas in Indonesia.

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Materials and Methods

Observation of morpho-agronomy characters and economic potential of Roid banana plants (*Musa* spp.) based on indigenous knowledge carried out in November 2021 - March 2022. This research was conducted in three villages in Jatigede District: Ciranggem Village, Jemah Village, and Mekarasih Village. The material used in this research is the population of Roid bananas (Musa spp.). The tools used in this study include the Global Positioning System (GPS), banana descriptors (IPGRI, UPOV, PPU), and devices for data processing (Laptop, GraphPad Prism 9 software, Origin Pro, and Plantix).

This descriptive study uses exploratory and survey methods as data collection techniques. The exploratory method is aimed at obtaining characteristic data of superior accession candidates for Roid bananas. While the survey method is intended to determine the scope of indigenous knowledge of the local community on the important value of the existence of Roid bananas. The descriptors and the attached questionnaire guide the exploratory and survey methods.

Collected data will be analyzed descriptively for further interpretation. Observation of Roid banana characters in the field refers to the list of characters listed in the descriptors (UPOV, 1989; IPGRI, 2006) and Guidelines for Implementation of Uniqueness, Uniformity and Stability Tests (PPU). Purposive sampling technique determined the sample exploration locations and sources of indigenous knowledge survey. Purposive sampling or judgment sampling is a technique that deliberately selects and determines the sampling locations according to the research needs (Tongco, 2007).

Results of data analysis obtained from exploration and survey at each location were carried out through quantitative analysis of vegetation and diversity levels. Vegetation analysis was conducted to determine the dominant vegetation type in a community. The parameter of Important Value Index (INP) can be used to express a measure of dominance by adding up the values of Specific Density (K), Relative Species Density (KR), Frequency (F), Relative Species Frequency (FR), Dominance (D) and Relative Dominance (DR). The formula used in calculating INP according to (Muller-Dombois and Ellenberg, 1976) is as follows: The seven arithmetic components are interrelated and have different functions. According to Prayoga et al. {2011), the function of each component is to describe the level of dominance of local bananas (*Musa* spp.) in an area. Analysis of the diversity level of the population of banana species was calculated using the Shannon-Wieners diversity index. According to Magurran (1988), an analysis of the level of biodiversity at the ecosystem level with the Shannon-Wieners diversity index was calculated using the following formula:

$$H = -\sum \frac{ni}{N} ln \frac{ni}{N} \text{ or}$$
$$H = -\sum pi \ln pi$$

description:
ni = Importance value of each type (individuals number of each type)
N = Total importance value (sum of all individuals)
Pi = Odds of interest for each type (ni/N)

The diversity index value (H) can be classified into four criteria: very high, high, medium, and low. The limits of the range of H values for each criterion according to Magurran (1988) are as follows: H> 3.0 indicates very high diversity, H 1.6 – 3.0 indicates high diversity, H 1.0 – 1.5 indicates moderate diversity, and H < 1.0 indicates low diversity.

Results and Discussion

Based on survey and exploration results Roid banana growing areas can be found in most parts of Jatigede District. Roid banana observation was conducted in six survey locations i.e. Ciranggem Village (Cikandang Hamlet and Ciranggem Hamlet), Jemah Village (Batugede Hamlet and Brujul Hamlet), and Mekarasih Village (Ciboboko Hamlet and Cihegar Mekar Hamlet). Roid banana observation sites belong to the lowland agro-ecosystem (<400 masl), with an altitude range between 295.2 masl to 364.8 masl. The range of temperature and humidity for banana planting locations in Jatigede District is 20-27^{o}C and 56-96%, respectively.

There are various types of plants in the Roid banana agroecosystem. Overall, there are 7

types of seasonal plants (rice, indigofera grass, leunca, cassava, eggplant and pumpkin) and 8 types of annual plants (mango, coconut, sugar palm, bamboo and cayenne pepper). The diversity of plant species in an agroecosystem can support the creation of a balance within an ecosystem (homeostasis). Homeostatic conditions in an area show that the ecosystem has the ability to adapt to changes that will occur in environmental conditions (Pusponegoro et al., 2018). The results of the analysis of the level of population diversity with the Shannon-Wieners index show that each village in Jatigede District has a different level of agro-ecosystem diversity. The biodiversity index of the banana agroecosystem in Jatigede District can be seen in Table 1.

Table 1. Index of diversity of banana agroecosystem species in Jatigede district

Locations	Index of Diversity	Criteria
Ciranggem Village	1.05	Medium
Jemah Village	0.50	Low
Mekarasih Village	1.48	Medium
Total	**3.03**	**High**

The index value of the diversity of agroecosystem species in Mekarasih Village and Ciranggem Village is included in the medium criteria. Meanwhile, Jemah Village has a low species diversity index. As for the whole of Jatigede District, it has a high species diversity index value of 3.03. This illustrates that the condition of the ecosystem at the observation site can support the growth of seasonal and annual plants in banana agroecosystems. Various types of plants found in banana agroecosystems will interact with each other, either directly or indirectly. According to Prayoga and Ismail (2020), most of the plant species found in the banana agro-exosystem in West Java did not have a significant negative effect on the growth of banana plants.

The diversity of Roid banana species is known by calculating the Importance Value Index (INP) which describes the position of a species relative to other species in a community. The greater the INP of a species, the higher the position and role of that species in a community. According to Safitria (2021), nine varieties of bananas were found in Jatigede District based on survey and exploration results. The nine types of bananas include Kapas, Roid, Raja Bulu, Ambon Hijau, Hurang, Nangka, Tanduk, Muli, and Raja Cere. The INP values of the nine varieties in Jatigede District can be seen in Figure 1. Roid bananas have an INP of 26.97% of all types of bananas in Jatigede District.

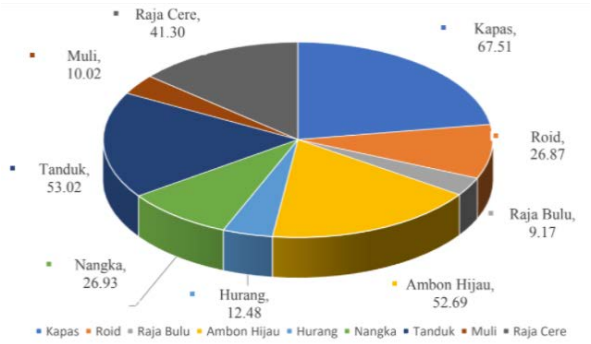

Figure 1. Combined INP of Nine Banana Varieties in Jatigede District
Source: (Safitria, 2021)

The calculation of the INP of Roid bananas at the research location is distinguished based on the location of its discovery. Ciranggem Village has the largest INP of Roid bananas among other villages, at 1.020 (34.01%). The INP of Roid bananas in Jemah Village was 1.016 (33.86%), while in Mekarasih Village, it was 0.964 (32.14%) (Figure 2). The variable that most influenced the IVI of Roid bananas in the three villages was relative density (KR). A high KR value indicates the density of Roid bananas against the density of all types of bananas in one area.

Cluster analysis classifies objects based on their homogeneity within the same cluster scope so it can increase the effectiveness of selection (Yuan et al., 2016). Cluster's formation for each group depends on the Euclidean distance. The euclidean distance from 18 Roid banana accessions results ranged from 4.5 to 7.4. This shows that Roid bananas in Jatigede District have wide variations. The kinship between accessions with an euclidean value of more than 1, shows a more distant kinship (Lestari and Julianto, 2020).

There are two parts of the dendrogram resulting from the cluster gram analysis, namely the accession dendrogram (row) and the morphological character dendrogram (column) (Figure 3). The column dendrogram depicts 38 morphological characters divided into two major

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groups with two subgroups each. Group I consisted of subgroup I with the characters of the number of rhizomes, the color of the underside of the leaf, the presence of male flowers, the position of the bunches, the diameter of the pseudostem, the diameter of the bunches, and the color of the peduncle. Subgroup II consisted of the width of the midrib, the opening of the bracts, the number of bunch combs, the curvature of the fruit, the length of the fruit stalk, the pattern of the bunches to the fruit, the width of the leaf blade, the diameter of the heart, and the number of fruits per comb. Group II consisted of subgroup I with the characters of pseudostem length, plant growth, heart shape, presence of flower organs on fruit, internal bracts color, pseudostem tapering, bunch stalk length, pseudostem anthocyanin staining, external bracts color, leaf blade length, and male flower attitude on rachis. While subgroup II consisted of pseudostem color, bunch shape, leaf base shape, bunch length, plant crown compactness, rachis: scars, bract: apex shape, inner color of the base of the pseudostem midrib, establishment of the base of the petiole wings, heart arch, and wax coating on the leaves.

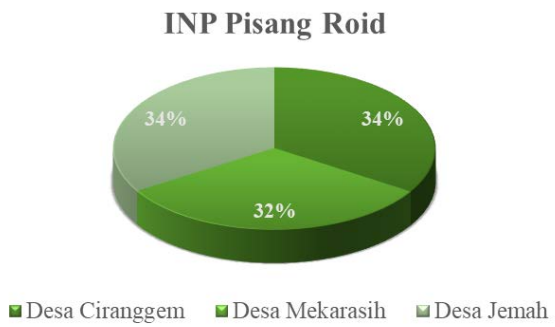

Figure 2. Combined INP of Roid Bananas at the Jatigede District Research Site

Line dendogram depicts the kinship of 18 Roid banana accessions which are divided into three major groups. Group I consisted of three accessions, namely MS1.3, MS1.2, and MS1.1. Group II consisted of 12 accessions, namely MS2.3, MS2.2, MS2.1, JM2.3, JM2.2, JM2.1, JM1.2, JM1.3, JM1.1, CGS2.3, CGS2. 2, and CGS2.1. Group III, consists of three accessions, namely CGS1.3, CGS1.2, and CGS1.1. The division of the three groups is based on the character of the establishment of the base of the petiole wings, and the male flowers: the opening of the bracts.

Each major group is divided into two subgroups. Subgroups of group I were classified based on differences in the number of rhizomes, the width of the midrib, the color of the underside of the leaf, and the length of the stalk. Subgroup I consisting of MS1.3 accessions had a higher number of tillers, wider frond margins, reddish green underside surface color, and longer stalk length (31-60cm) compared to subgroup II. The accessions in subgroup II included MS1.2 and MS1.1. In group III, the subgroups were differentiated based on these characters' bunch pattern to the fruit, the width of the leaf blade, and the attitude of the male flowers to the rachis. Subgroup I in group III had a bunch pattern of fruit that did not appear much, wider leaf blades, and male flowers were curved more than subgroup II. The accessions included in subgroup I was CGS1.3, while accessions in subgroup II were CGS1.2 and CGS1.1.

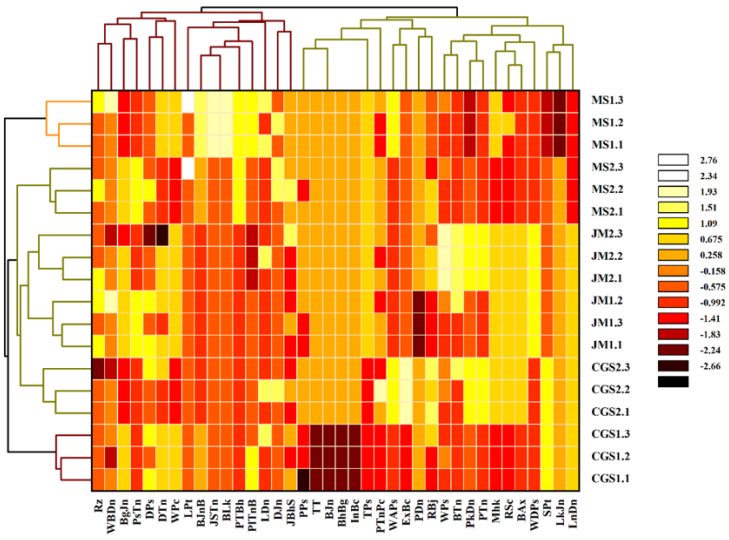

Figure 3. Clustergram Analysis with Heatmap Concepts on Morphological Characteristics of 18 Banana Roid Accessions in Jatigede District

Group II had the highest number of accessions among the other groups. Subgroups in group II were distinguished based on these characteristics: waxy coating on the leaves, compactness of the plant crown, scars on the rachis, shape of the apex on the bracts, opening of the bracts, and length of the fruit stalk. Subgroup I consisted of accessions MS2.3, MS2.2, and MS2.1 with the characters of slightly waxy leaves, looser compactness of plant crowns, weak ex scars on rachis, truncate shaped

apex bracts, fairly exposed bracts, and longer fruit stalk (≥21mm) compared to subgroup II. Sub-group II is further divided into two subgroups. Sub-subgroup I consisted of JM2.3, JM2.2, JM2.1, JM1.2, JM1.3, and JM1.1 accessions. Sub-subgroup I characters have greener inner Pseudostem, medium tapering pseudostem, petiole wings base is no deeper than Sub-subgroup II, which consists of accessions CGS2.3, CGS2.2, and CGS2 .1.

Clustergram analysis with the concept of a heat map dendrogram illustrates the influence of characters on variation between accessions with differences in color intensity. Characters that have extreme bright colors are characters that have a significant effect on the differences between accession clusters. The brighter the color of the accession group to the characters, the higher the euclidean value between the two variables (Anshori et al., 2018). Characters showing extreme color intensity on the heat map include midrib width (LPt), bunch diameter (DTn), and pseudostem length (PPs) with an average euclidean value of > 1.93 and < -2.24.

Conclusion

1. Roid bananas (*Musa* spp.) in Jatigede District have wide genetic diversity and distant genetic kinship with a euclidean value of 4.5-7.4.
2. Germplasm that has a superior potential accession for Roid banana (*Musa* spp.) based on the identification of genetic diversity and kinship is MS1.3 accession. Accession MS1.3 characters are a large number of combs, leaf blade width of 71-80 cm, leaf blade length of 171-220 cm and a number of rhizomes > 5 tillers which have potential as genetic resources to produce new superior varieties.

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Effect of NPK fertilizer dose and GA_3 concentration on growth, yield, and yield quality of *Coix lacryma-jobi* L. var. *ma-yuen* from ratoons

Abstract. One type of cereal that can be used as a functional food is hanjeli (*Coix lacryma-jobi* L.). Hanjeli production has many problems, i.e., low productivity and long life. Hanjeli cultivation from ratoons has several advantages, including reduced production costs, shorter lifespan, and less water requirement. Fertilization of NPK and application of GA_3 as gibberellin hormone are expected to increase the growth and yield of hanjeli. This study aims to determine the interaction effect between NPK and GA_3 on the growth and yield of hanjeli from ratoon. This experiment was carried out at the Experimental Field of the Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java, from August to December 2020. The experimental design used a Randomized Block Design, while the treatment design consisted of 2 factors. The first factor was the dose of NPK fertilizer which consisted of 3 levels, namely 100, 200, and 300 kg. The second factor was the concentration of GA_3, consisted of 3 levels, namely 0, 10 and 20 ppm. All treatments were repeated 3 times. The results showed that there was an interaction effect between NPK fertilizer application and GA_3 concentration on growth and yield of hanjeli, namely plant height, number of tillers, number of branches, leaf area index, number of productive tillers, grain weight per plant, harvest index and grain size and hardness. At a high concentration of GA_3, increasing NPK fertilizer dose could improve the growth, yield, and yield quality of the hanjeli.

Keywords: Fertilizer · Functional food · Gibberellin · Ratoon

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Introduction

Hanjeli (*Coix lacryma-jobi* L.) is a cereal plant that is used as an alternative food. Hanjeli has been used to make indigenous porridge for a long time, especially in West Java, Southeast Sulawesi and South Sulawesi. According to Nurmala (2010), hanjeli can be used as an ingredient in brownies and other cakes. Hanjeli also has various health benefits (bin Arif et al., 2014; Afandi et al., 2019; Tensiska, 2017; He et al., 2020; Tensiska et al., 2020; Grubben et al., 1996). One widely cultivated hanjeli variety is *Coix lacryma-jobi* var. *ma-yuen* (Qosim & Nurmala, 2011).

Currently, the hanjeli plant has many problems, namely low productivity and long life. The hanjeli cropping index can be increased by ratooning the plants, thereby shortening the land preparation time and the life of hanjeli plants (Mareza et al., 2016). This is because ratoons can grow new plants by growing shoots from the base of the stem after the plants are cut (Efendi et al., 2013). However, ratoons of hanjeli cause decreasing crop yields, even more than 50%.

To keep decreasing yield not significant, a booster is needed to accelerate hanjeli growth. NPK fertilizer is needed for plant growth. Nitrogen (N) is necessary for the production of carbohydrates, proteins, lipids and other organic substances and is also a component of chlorophyll, which gives green color of leaves (Wang et al., 2021). Phosphorus (P) helps energy transfer in plant cells, encourages root development and early fruiting, strengthens stems to prevent them from breaking, and increases absorption at the start of growth (Balemi & Negisho, 2012). Potassium (K) plays roles in plant growth, for example in encouraging the translocation of carbohydrates from leaves to plant organs (Assagaf, 2017).

On the other hand, gibberellins are known to increase plant growth, both vegetative and generative (Sarwanidas & Setyowati, 2017). Gibberellin is a plant growth regulator which plays a role in stimulating stem segment elongation and increasing leaf size in various plants, which increasing cell elongation and expansion is one of the effects of gibberellins (Wicaksono et al., 2016; Schwechheimer, 2012).

The enlarged cell size as a result of gibberellins will require additional nutrient supply (Ullah et al., 2017; Saleem et al., 2021). The addition of NPK and gibberellin is expected to increase the growth of hanjeli plants, so that the yield and quality of the results will increase. This study aims to determine the interaction effect of NPK fertilizer and gibberellin (GA_3) on the growth, yield, and quality of yield of *Coix lacryma-jobi* L. var. *ma-yuen* from ratoon.

Materials and Methods

This research was conducted from August to December 2020 at the Experimental Field of the Faculty of Agriculture, Padjadjaran University, Jatinangor, Sumedang Regency, West Java. The altitude of the research location is around 737 m asl, the soil order is Inceptisols and climate type is C3 according to Oldeman's classification. The tools used in this study was a meter gauge, analitycal scale with 0.01 gram accuracy, camera, Irfan view software, huller machine, caliper, and penetrometer. The materials used are the seeds of hanjeli var. *ma-yuen*, plant growth regulator GA_3 10%, NPK 16 – 16 – 16 fertilizer, and Profenofos insecticide.

This research used experimental method. The experimental design used factorial Randomized Block Design (RBD) consisting of 2 factors: NPK fertilizer dose as the first factor and gibberellin concentration (GA3) as the second factor. The first factor consisted of 3 levels, namely 100 (p1), 200 (p2), and 300 (p3) kg ha^{-1}, while the second factor consisted of 3 levels, namely 0 (g0), 10 (g1), and 20 (g2) ppm . All treatments were repeated three times. The samples observed for each plot were 6 plants.

Observations were made on growth characteristics (plant height, number of tillers, number of branch and leaf area index), yield components (number of productive tillers, grain weight per plant and harvest index), and yield quality (grain size and grain hardness). Plant height was measured using a meter gauge at the end of the vegetative stage (14 weeks after ratooning). The number of tillers and the number of branches were counted at the age of 14 weeks after ratooning. The leaf area index was measured by comparing the leaf area with the canopy area. Leaf area was measured using the regression equation $y = 0.277 + 0.68$ (lxw) ($R^2 = 94.5\%$), where l is leaf length while w is the leaf width. Canopy area was measured using a photo of area covered by plants, that converted into pixel by Irfan view software. The number of

productive tillers is counted based on the number of tillers that produce flowers at the reproductive stage. Grain weight per plant was measured after harvest using an analytical scale at 14% moisture content. The harvest index is measured by comparing grain weight with plant biological weight. Grain size was measured using a caliper, while grain hardness was measured using a penetrometer.

Experimental data were analyzed using the ANOVA test. Differences at each level were analyzed using Duncan's test at a significant level of 5%.

Results and Discussions

Results. Tables 1 and 2 showed the interaction effect between NPK fertilizer doses and gibberellin (GA_3) concentrations on plant height and number of tillers. In the observation of plant height, doses of p3 and p2 gave higher plant height than p1 at the g0 and g1 levels, but the p2 treatment was not different from p1 at the g2 level. Meanwhile, in observing the number of tillers at the g0 level, the p1 and p2 doses were not different, although they differed from the p3 dose. At the g1 and g2 levels, all treatments gave different number of tillers. The increasing number of tillers occurred with the addition of gibberellin concentrations and NPK fertilizer.

Table 1. Interaction effect of NPK fertilizer and gibberellin concentration on plant height (cm)

NPK Fertilizer (P)	Gibberellin (G)		
	g_0 (0 ppm)	g_1 (10 ppm)	g_2 (20 ppm)
p_1 (100 kg ha^{-1})	136.44 a A	135.89 a A	144.83 a B
p_2 (200 kg ha^{-1})	162.50 b B	155.44 b AB	150.50 a A
p_3 (300 kg ha^{-1})	155.56 b A	154.28 b A	174.44 b B

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Likewise with the number of branches and leaf area index, an increase in the number of branches or leaf area index occurred at high concentrations of gibberellin with the addition of NPK fertilizer. The number of branch and leaf area index at the g0 level showed no difference in the dose of fertilizer treatment, but the p3 dose gave a different number of branch or leaf area index with the doses of p1 and p2 at the g1 and g2 levels (Tables 3 and 4).

Table 2. Interaction effect of NPK fertilizer and gibberellin concentration on number of tillers

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	13.33 a B	10.06 a A	17.06 a C
p_2 (200 kg ha^{-1})	13.11 a A	14.11 b A	18.33 b B
p_3 (300 kg ha^{-1})	17.56 b A	19.11 c B	24.56 c C

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Table 3. Interaction effect of NPK fertilizer and gibberellin concentration on number of branch

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	3.17 a A	3.22 a A	4.33 a B
p_2 (200 kg ha^{-1})	3.28 a A	3.28 a A	4.22 a B
p_3 (300 kg ha^{-1})	3.33 a A	4.22 b B	5.28 b C

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Table 4. Interaction effect of NPK fertilizer and gibberellin concentration on leaf area index

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	3.212 a B	2.190 a A	3.845 a B
p_2 (200 kg ha^{-1})	2.532 a A	2.705 a A	4.183 a B
p_3 (300 kg ha^{-1})	3.437 a A	5.182 b B	7.791 b C

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

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In observing the yield characters, there was an interaction effect between NPK fertilizer and gibberellin on the number of productive tillers (Table 5). At the g0 level, the p1 and p2 doses did not differ, although they were different from the p3 dose. All treatments were different at the g1 and g2 levels. The same pattern occurs as in the observation of the number of tillers.

Table 5. Interaction effect of NPK fertilizer and gibberellin concentration on number of productive tillers

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	12.06 a B	9.44 a A	14.00 a C
p_2 (200 kg ha^{-1})	11.06 a A	11.56 b A	17.00 b B
p_3 (300 kg ha^{-1})	17.44 b A	17.56 c A	20.61 c B

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Other yield characters were also affected by the interaction between NPK fertilizer and gibberellin, one of which is grain weight and harvest index. The NPK fertilizer treatment did not affect grain weight at the g1 and g2 levels, but the p3 dose gave the best seed weight at the g3 level. Similarly to the observation of grain weight, NPK fertilizer treatment increased the harvest index at high doses of gibberellins (Tables 6 and 7).

Table 6. Interaction effect of NPK fertilizer and gibberellin concentration on grain weight (g)

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	51.22 a A	60.41 a B	68.27 b B
p_2 (200 kg ha^{-1})	52.84 a A	58.01 a A	51.66 a A
p_3 (300 kg ha^{-1})	57.38 a A	61.99 a A	98.82 c B

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Table 7. Interaction effect of NPK fertilizer and gibberellin concentration on harvest index

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	0.22 a A	0.26 a B	0.21 a A
p_2 (200 kg ha^{-1})	0.23 a B	0.26 a B	0.19 a A
p_3 (300 kg ha^{-1})	0.22 a A	0.24 a A	0.34 b B

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Table 8. Interaction effect of NPK fertilizer and gibberellin concentration on grain size (mm)

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	5.22 a A	6.76 a B	7.04 a B
p_2 (200 kg ha^{-1})	6.90 b A	6.90 a A	7.05 a A
p_3 (300 kg ha^{-1})	7.40 b AB	7.10 a A	7.84 b B

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

Table 9. Interaction effect of NPK fertilizer and gibberellin concentration on grain hardness (kgF)

NPK Fertilizer (P)	Gibberellin (G)		
	g_1 (0 ppm)	g_2 (10 ppm)	g_3 (20 ppm)
p_1 (100 kg ha^{-1})	4.42 a A	4.47 a A	4.75 a B
p_2 (200 kg ha^{-1})	4.46 a A	4.63 b B	5.08 b C
p_3 (300 kg ha^{-1})	4.55 a A	5.06 c B	6.15 c C

Note: The mean value followed by the same capital letter (horizontal direction) or the same lowercase letter (vertical direction) is not significantly different according to Duncan's test at 0.05 significant level

In observing the yield quality, NPK and gibberellins had an interaction effect on grain size, but this did not appear to be consistent (Table 8). The increase in grain size at the g1 level was due to

p2 and p3 doses, but all NPK doses did not differ at the g2 level, then the p3 dose increased seed size again at the g3 level. On the other hand, increasing NPK fertilizer increased grain hardness at high doses of gibberellins (Table 9).

Discussion. The effect of gibberellin concentration occurred on growth, yield, and yield quality. This can happen because gibberellins can increase cell enlargement (Schwechheimer, 2012). Gibberellins are known to increase plant growth, both vegetative and generative (Sarwanidas & Setyowati, 2017). Gibberellins can increase the height and number of tillers of cereal plants (Wicaksono et al., 2016; Maharani et al., 2018). Gibberellins can also increase leaf area (Wicaksono et al., 2016). According to Pratama's research (2019), the GA_3 treatment had a significant effect on the weight of edamame seeds. The addition of GA_3 at the beginning of the seed formation process can accelerate cell proliferation and expansion, resulting in larger seeds and increased seed weight (Yasmin et al., 2014). Putra et al.'s research (2014) stated that giving gibberellins (GA_3) increased the harvest index on soybeans. Sriyanto et al. (2019) and Rasyad & Nurbaiti (2014) stated that the application of GA_3 resulted in a larger size and harder seed hardness.

Based on the results of the study, increased growth, yield, and yield quality through higher application concentrations of GA_3 were obtained at higher doses of NPK fertilizer as well. The increase in growth and yield causes the supply of nutrients to be increased. According to Wahyudi et al. (2012), the more adequate the dose of fertilizer given, the better the effect on growth because plants will not be able to grow and develop properly if the nitrogen, phosphorus and potassium needed are insufficient.

Increases in growth, yield, and yield quality in cereal crops have been widely reported as a result of NPK fertilization. The number of tillers of paddy rice increases with higher NPK fertilization (Mahmud, 2015). Research by Pusparini et al. (2018) and Assagaf (2017) stated that the growth and yield of maize plants showed optimum results with higher NPK doses. The elements of nitrogen, phosphorus, and potassium can affect the formation of hanjeli branch (Ruminta et al., 2017). According to Murtilaksono et al. (2014), the number of branch and panicles that grow is due to the large number of tillers that grow and are formed due to excess photosynthate. Nitrogen nutrients affect the leaf area index (Irwan & Nurmala, 2018). Yelis (2011) stated that the elements P and K are needed for the formation of hanjeli grains which causes the grains to be fuller. Kurniadie's research (2002) showed that applying NPK fertilizer to lowland rice at a dose of 300 kg/ha gave the highest average harvest index.

Conclusion

There were interaction effect between NPK fertilizer dose and GA_3 concentration of *Coix lacryma-jobi* L. var. ma-yuen produced from ratoons on plant height, number of tillers, number of branch, leaf area index, number of productive tillers, grain weight, harvest index, grain size and hardness.

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