

# Jurnal KULTIVASI

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## **PREFACE**

The articles included in this volume 23, issue 1 (2024) of Kultivasi Journal come from various scientific backgrounds related to agriculture. These articles about plant production are from multiple fields, including soil science, weed science, plant pests and diseases, agronomy, and plant breeding. Plant output is increased by this knowledge, both commercially and in the early stages.

The 14 articles from academicians and researchers in this publication have still been edited and reviewed by experienced editors and reviewers from Indonesia and overseas. These articles are anticipated to broaden the academic understanding of agricultural practitioners and academic scientists. The future growth of the farming sector depends heavily on the involvement of scholars and researchers. This year, Kultivasi Journal has consistency and routinely released an English edition. It will prepare and encourage our journal to be indexed in International scope for the next.

Thank you for the loyalty of the reader, author, and reviewers in continually developing our publishing.

Editorial of Kultivasi Journal

## AUTHOR'S INSTRUCTIONS

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

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

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

### Special Requirements

Review Articles:

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

The systematics of writing peer articles consists of: Title, author's name and correspondence address; Abstract with

keywords; The Introduction contains justifications for the importance of the topic being discussed; Subject matter; Conclusion; Acknowledgment; and References.

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Research Articles:

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

Title

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

Author's name

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

Abstract

- Abstract is an informative writing that is a brief description about the background, objectives, methods, results and conclusions. Abstract is written in English

with a maximum of 250 words and equipped with keywords.

#### Introduction

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

#### Materials and Method

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

#### Results and Discussions

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

#### Conclusion

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

#### Acknowledgment

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

#### Reference

There are at least 20 references from the last 10 years. The references list all related libraries

along with the aim of making it easier to search for readers who need it. Only list libraries that have been published either in the form of textbooks or scientific articles. Using an internationally applicable article author's name writing system. Inside the text, the reference should be written as follows:

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Suminar E · Rahmawati V · Sumadi · Nuraini A

## ***In vitro* multiplication of strawberry in various types and concentrations of cytokinins and auxins**

**Abstract.** Growth regulator is one of the important factors in the success of plant propagation by tissue culture. 6-Benzylaminopurine (BAP) is one of the most widely used cytokinin because it is more stable, less expensive and more effective. Thidiazuron (TDZ) can increase the action of other cytokinins, either exogenous cytokinin or endogenous cytokinin. 1-Naphthaleneacetic acid (NAA) belongs to the more stable auxin group, because it is not easily decomposed and oxidized by enzymes released by plants. This study aimed to obtain the best types and concentrations of BAP, TDZ, and NAA for the *in vitro* multiplication of strawberry explant. The experiment was conducted at the Seed Technology and Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran, from April to July 2022. The explants used were axillary shoots of strawberry var. Sweet Charlie is derived from plantlets that were propagated *in vitro*. The experimental design used was a completely randomized design with six treatments and four replications. The media used were Murashige dan Skoog (MS) with the addition of plant growth regulators (PGRs) in the form of BAP (0 ppm and 0,5 ppm), TDZ (0 ppm and 0,1 ppm), and NAA (0 ppm and 0,1 ppm). The application of different types and concentrations of BAP, TDZ, and NAA resulted in different effects of *in vitro* shoot multiplication of strawberry explant var. Sweet Charlie. Concentration of 0.1 ppm TDZ produced the highest number of shoots (5.38).

**Keywords:** BAP · NAA · Strawberry · TDZ · Tissue culture

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## Introduction

Strawberry (*Fragaria x ananassa*) is one of the fruit plants with high economic value and greatly demanded by people around the world. It has high nutritional and antioxidant content because its quercetin, ellagic acid, anthocyanins, and kaempferol content (Ministry of Agriculture of the Republic of Indonesia, 2019). Cultivated strawberries in Indonesia are frequently resulted from introductions, i.e., cv. Sweet Charlie from the United States of America (USA).

The demand for strawberries in Indonesia is quite high, but it is not accompanied by the level of production. Production of strawberries in Indonesia continues to decline. Strawberry's production in 2020 was 8,350 tons, a decrease from 2017 at 12,225 tons and 2018 at 8,531 tons (Statistics Indonesia, 2020).

Strawberry plants are susceptible to disease caused by viruses that infect plants, this is one of the main factors causing a decrease in strawberry yields of up to 80% (Research Institute for Citrus and Subtropical Fruit Crops, 2015; Thompson & Jeikman, 2003). Apart from that, powdery mildew fungus infection can reduce the productivity of strawberry plants in producing fruit (Wahyuni, 2016). The system for procuring strawberry seeds with conventional methods, both generative and vegetative (with stolons), is still less effective in producing superior and disease-free seeds because it has the potential to transmit congenital diseases from parental plants (Widiastuti, 2015).

One of the methods to provide strawberry seeds in large quantities that are free of disease is by tissue culture technique. The tissue culture technique has several advantages compared to other conventional techniques, such as being able to produce seeds on a large scale, being uniform, healthy, or free of pathogens, and not requiring large areas of propagation land (Hardiyati et al., 2017).

The shoot multiplication stage is one of the important stages in tissue culture technique. The addition of PGRs to the culture medium can improve plant multiplication ability in tissue culture (Wahyuni et al., 2020). According to Lestari (2011), PGRs are regularly used in the culture medium in tissue culture are types of auxins and cytokinins whose use depends on the purpose or direction of the desired plant growth. Comparison of the appropriate concentrations of PGRs of auxin and cytokinin in tissue culture is

known to stimulate a faster shoot multiplication process.

Bimantara (2018) who work with strawberry cv Earlibirte meristem culture, showed that the treatment of 0.5 ppm BAP + 0.025 ppm NAA gave the best results on the number of shoots and number of leaves. The addition of 0.50 ppm BAP has a good effect on the number of shoots, number of leaves, plantlet fresh weight, and runner, while the excessive use of TDZ gave defective results on the growth of shoots, leaves, and roots of strawberry plantlets cv Tochiotome (Raisya et al., 2020).

The addition of cytokinins in the form of BAP or TDZ which plays a role in stimulating shoot growth and cell division, as well as auxin in the form of NAA which plays a role in stimulating root growth and cell elongation, are expected to have a positive effect to improve strawberry multiplication ability. The information about the role of BAP, TDZ, and NAA in the *in vitro* shoot multiplication stage in strawberry is still not widely known, therefore it is necessary to research the type and concentration of cytokinins (BAP and TDZ) and auxin (NAA), for the *in vitro* shoot multiplication of strawberry explants var Sweet Charlie.

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## Materials and Methods

The research was conducted from April to July 2022 at the Seed Technology and Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The tools used in this study include culture bottles, tweezers, scalpels, Laminar Air Flow (LAF), petri dishes, etc. The plant material used in the present experiment was explant in the form of axillary shoots aged 23 days after planting (DAP) from the 5<sup>th</sup> subculture of the strawberry var Sweet Charlie plantlet that was propagated *in vitro*. Other materials used were BAP, TDZ, and NAA.

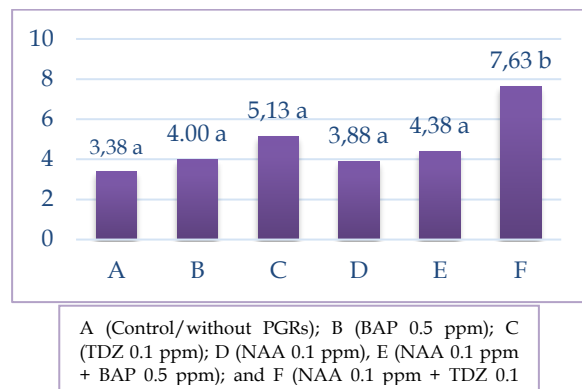
The study was designed with a completely randomized design consisting of six treatments, repeated four times. The multiplication medium was MS base media with 6 treatments of PGR addition, i.e., A (Control/without PGR); B (BAP 0.5 ppm); C (TDZ 0.1 ppm); D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP); and F (0.1 ppm NAA + 0.1 ppm TDZ).

Observational parameters include shooting emergence time (DAP), number of shoots, number of roots, and fresh weight of plantlets.

Obtained data was analyzed by analysis of variance at 5% and If there is a significant difference, it will continue by the Tukey test. All statistical analysis was performed in SPSS 28.0 software.

## Results and Discussion

**Shooting Emergence Time.** The results of the analysis of variance showed that the addition of cytokinins and auxin had a significant effect on the average time of shoot emergence. According to Kurnia (2014), the addition of exogenous hormones can cause stimulation similar to phytohormones that are naturally produced by plants, so they could affect plant growth and development in the process of cell division, enlargement, and differentiation.



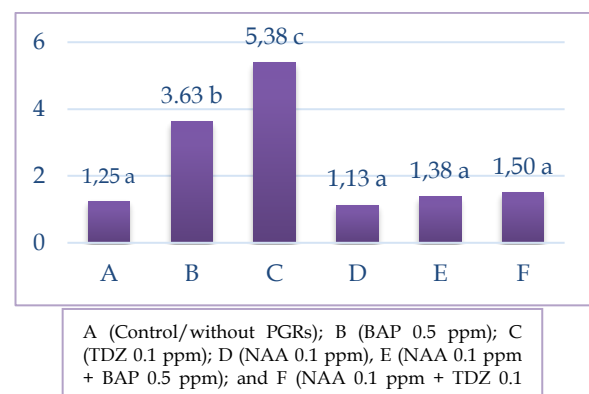
**Figure 1. Shooting emergence time (DAP)**

The experimental results showed that the shoot emergence time in treatment F (0.1 ppm NAA + 0.1 ppm TDZ) was significantly different from treatment A (Control), B (0.5 ppm BAP), C (0.1 ppm TDZ), D (0.1 ppm NAA), and E (0.1 ppm NAA + 0.5 ppm BAP).

Treatment F (0.1 ppm NAA + 0.1 ppm TDZ) gave the highest average shoot emergence time (7.63) compared to other treatments (Figure 1). The addition of exogenous auxins such as NAA is known to stimulate the production of ethylene hormone, especially in high concentrations. The addition of TDZ singly or in combination can inhibit the emergence of shoots, Sjahril et al. (2016) assumed that TDZ with a certain concentration can produce a compound in explants that can slow down the time of shoot emergence. Guo et al. (2011) further explained that TDZ can stimulate the production of the

ethylene hormone. According to Zulkarnain (2009), the ethylene hormone in tissue culture can inhibit the process of morphogenesis and shoot growth, so the addition of TDZ either singly or in combination will inhibit the emergence of shoots. TDZ is also involved in the process of auxin synthesis by increasing levels of endogenous auxin, namely indole-3-acetate (IAA) (Cappelletti et al., 2016), so it can be assumed that treatment F (0.1 ppm NAA + 0.1 ppm TDZ) induces more ethylene hormone than other treatments, so it can inhibit shoot formation and result in a longer shoot time.

**Number of Shoots.** The results of the analysis of variance showed that the addition of PGRs had a significant effect on the average number of shoots at 12 WAP.



**Figure 2. Number of shoots at 12 WAP**

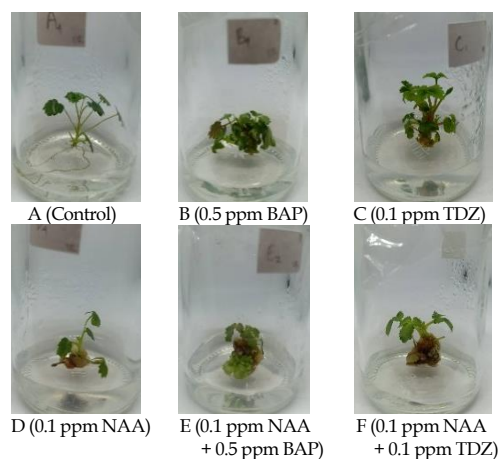
Based on Figure 2, treatments A (Control), D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) produced shoots that were not significantly different, but significantly different from treatments B (0.5 ppm BAP) and C (0.1 ppm TDZ) at 12 WAP. The average number of shoots in treatment C (0.1 ppm TDZ) was significantly different from treatment B (0.5 ppm BAP), this was presumably due to the addition of 0.1 ppm TDZ can stimulate shoot growth faster than the addition of 0.5 ppm BAP.

The addition of TDZ in low concentrations (0-0.50 ppm) can stimulate the growth of axillary shoots of the strawberry cv Tochiotome, while at high concentrations (more than 0.50 ppm) for a long time can cause abnormality to the explant tissue thereby reducing the ability of shoot regeneration (Raisya et al., 2020), so that it can cause a decrease in shoot micropropagation and inhibit shoot elongation. In the study of



Cappelletti et al. (2016), it is known that the addition of 0.50 ppm TDZ produced the highest number of shoots (3.60) with an indirect organogenesis process in blueberry cv Duke, while in the research of Raisya et al. (2020), the addition of 0.25 ppm TDZ resulted in the lowest number of shoots (1.70) in strawberry cv Tochiotome.

In this study was found that treatment C with the addition of 0.1 ppm TDZ (Figure 2) resulted in the highest average number of shoots (5.38) at 12 WAP. According to Kusmianto (2008), TDZ can stimulate the production of endogenous cytokinins, and can also act as an inhibitor of cytokinin oxidase which is an enzyme that can eliminate the activity of free adenine-type cytokinins. Therefore, it can be assumed that the addition of 0.1 ppm TDZ singly can increase the production and activity of endogenous cytokinins to induce shoot growth to produce more plantlet shoots.



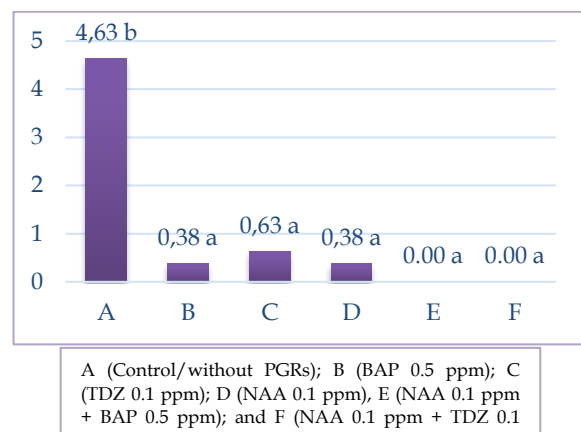
**Figure 1. Plantlet form at 12 WAP**

Treatment D (0.1 ppm NAA) produced the lowest number of shoots (1.13), but it was not significantly different from treatments A (Control), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) at 12 WAP (Figure 2). The low value of the average number of shoots was thought to be due to the addition of PGR in the form of auxin (NAA), which was less effective in promoting the growth of strawberry plantlet shoots. This is in accordance with the research of Sjahril et al. (2016), that the addition of NAA was less effective in stimulating shoot growth and only produced 2.00 shoots with the addition of 0.5 ppm NAA, while the addition of 0.1 ppm and 2.0 ppm NAA did not produce plantlet shoots at all. This was presumably that the addition of auxin to the

culture medium can inhibit the growth of axillary shoots in explants (Sjahril et al., 2016).

Strawberry plantlets growing at 12 WAP in treatments B (0.5 ppm BAP) and C (0.1 ppm TDZ) looked denser because they formed a rosette (Figure 3), this was due to a large number of branches and leaves growing on the explants. Rantau et al. (2021) explained that the active growth of new shoots could inhibit the growth of shoot height, causing thicker stems and a more rosette shape of explants. This is presumably that the addition of BAP or TDZ singly in the medium can result in nutritional competition for explants in culture medium due to the higher number of branches and leaves of explants (Danial et al., 2016).

**Number of Roots.** Based on the results of the analysis of variance, it was found that the PGRs treatment had a significant effect on the average number of plantlet roots of strawberry plants at the end of the observation time (12 WAP). The addition of BAP, TDZ, and NAA resulted in few, short, and no roots.

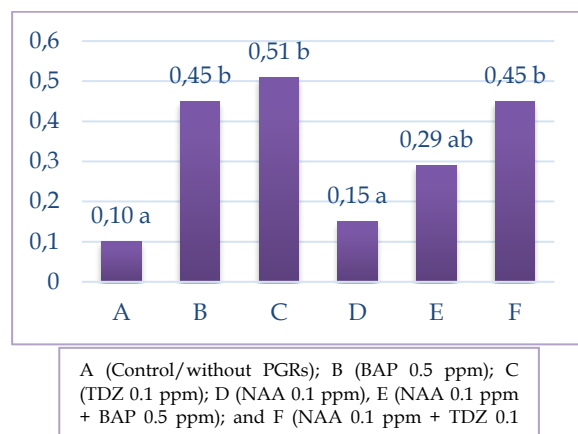


**Figure 4. Number of roots at 12 WAP**

The number of roots in treatments B (0.5 ppm BAP), C (0.1 ppm TDZ), D (0.1 ppm NAA), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ) was not significantly different, but significantly different from treatment A (Control). Strawberry explants were grown on medium with the addition of cytokinins and auxins only produced small amounts of roots and did not even produce roots. Rantau et al. (2021) explained that plant explants were less able to form roots in culture medium with the addition of cytokinins and auxins because the addition of exogenous cytokinins and auxins could inhibit the biosynthesis process of endogenous auxin in

forming roots in plant explants. Treatment A (Control) produced the highest number of roots, which was 4.63 (Figure 4). This is in accordance with the research results of Raisya et al. (2020), that the highest number of roots was found in treatment A (without PGRs) which was 1.20 at 4 WAP and 2.70 at 8 WAP.

Plantlet roots were able to grow even though the culture medium was not given additional PGRs in the control treatment, it was presumably that the endogenous auxin content in the explants was quite high so that it could stimulate the formation of plantlet roots even though no PGRs was added (Murti et al., 2012; Haddadi et al., 2010). Plants can naturally produce the auxin hormone even though it is produced in small amounts (Mutmainah, 2016). The addition of a combination of auxin and cytokinin in treatments E (0.1 ppm NAA + 0.5 ppm BAP) and F (0.1 ppm NAA + 0.1 ppm TDZ) was presumably to inhibit root growth but stimulate callus growth due to hormonal balance in the culture.



**Figure 5. Fresh weight of plantlets (g)**

**Fresh Weight of Plantlets.** The results of the analysis of variance showed that the addition of PGR to the culture medium had a significant effect on the average number of plantlet fresh weight (Figure 5). This proves that the explants can absorb the nutrients contained in the culture medium. Nutrients that have been absorbed by explants are used in the growth process, such as to form shoots, leaves, and roots. The yield of plantlet fresh weight depends on the speed of cell division, cell multiplication, and shoot enlargement (Sulasiah et al., 2015).

Based on Figure 5, it is known that the average number of fresh weight in treatments B (0.5 ppm BAP), C (0.1 ppm TDZ), and F (0.1 ppm NAA + 0.1 ppm TDZ) did not significantly different from treatment E (0.1 ppm NAA + 0.5 ppm BAP), but significantly different from treatments A (Control) and D (0.1 ppm NAA). The highest average yield of plantlet fresh weight (0.51 g) was found in treatment C (0.1 ppm TDZ), although it was not significantly different from treatments B (0.5 ppm BAP), E (0.1 ppm NAA + 0.5 ppm BAP), and F (0.1 ppm NAA + 0.1 ppm TDZ). This is in accordance with the research by Raisya et al. (2020), that the addition of TDZ on culture medium resulted in a large fresh weight of strawberry plantlets (0.50 g), but it was not significantly different from the addition of BAP. This is thought to occur because the addition of TDZ can affect the level of accumulation of minerals or other metabolites (such as amino acids, nucleotides, sugars, lipids, and other compounds) which are relatively high in plant tissues (Angin et al., 2019). That is the response of plant explants to stress and the effects of TDZ to produce thicker shoots (Raisya et al., 2020), so it can affect the yield of plantlet fresh weight.

## Conclusions

Based on the results of the study, it can be concluded that:

1. Different types and concentrations of BAP, TDZ, and NAA produced different effects on *in vitro* shoot multiplication of strawberry var Sweet Charlie explants.
2. The addition of 0.1 ppm TDZ resulted in the highest number of strawberry plantlet shoots (5.38).

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Shakina VD · Nuraini A · Nurbaity A

## Response of tomato seed germination to several extraction techniques and magnetic field exposure treatments

**Abstract** The superior seeds are indicated by clean, good germination rate, pest and disease-free. One of the problems in providing superior tomato seeds is the presence of pulp attached to the tomato seeds, leading to less clean of seed. To remove the mucus on tomato seeds, the seeds have to be extracted. Other than seeds extraction, seeds exposure also could optimize seeds growth by increasing the metabolism of the tomato. The aim of this research is to determine the best extraction technique and magnetic field exposure for obtaining high tomato seeds germination. The complete random design used in this research consist of two factors, seeds extraction and magnetic field with three time replications. The result showed that seeds extraction for 24 hours is the best treatment for improving the maximum germination percentage, growth potential, growth rate, speed growth, uniformity index than others. Magnetic exposure at 6mT is the best treatment to improve seeds maximum growth and seeds speed growth.

**Keywords:** Extraction · Germination · Magnetic field · Tomato

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## Introduction

Tomato which is one of the five vegetable crops in Indonesia which contributes the largest production after chili is a fruit vegetable with high economic value. Tomato production in 2020 reached 1.08 million tons, while tomato consumption in 2020 reached 1.4 million tons (Statistics Indonesia, 2020). Optimizing tomato production in the field is still constraint low quality seed, pests and diseases, low percentage of fruitset, uncertain climatic conditions, and soil fertility. These constraints cause a decrease in tomato production, both in terms of quality and quantity, even if it is not immediately anticipated, it can cause crop failure (Hidayati & Dermawan, 2012).

Superior seeds are clean and healthy seeds, and also have good germination. One of the problems in providing tomato seeds is because of the pulp on the tomato seeds. To remove the pulp on the tomato seeds, the seeds needed to be extracted. If it is not extracted well, it would cause germination of the old seeds, easily contaminated by the microbes, and affect the vegetative and generative growth (Rismunandar, 2001).

Unclean tomato extraction could be characterized by the existence of juice and pulp on the tomato seeds and the physical appearance that looks yellow and dull (Karavina et al., 2009). There are several wet extraction techniques such as soaking with fermented tomato water, HCl solution, and NaOCl solution. Iriani et al. (2017) proved that the extraction technique with fermented tomato water for 24 hours resulted in a simultaneous growth of 91%, 91% germination, and normal dry sprout weight for 0,11 grams. This research also proved that the extraction technique with 2% HCl solution for 2 hours produced a 98.25% simultaneous growth, 98.25% germination rate, and 0.11 g normal dry sprout weight.

Other than fermented tomato water and HCl solution, NaOCl solution also could be used for the wet extraction technique. Purba et al. (2018) proved that the extraction technique with 9% NaOCl solution produced higher germination, seed growth rate, and maximum growth potential rather than soaking in regular water, 6% and 13% NaOCl. According to Gunarta (2014), tomato seed extraction treatment by soaking in 2% HCl solution for 2 hours showed the best average value for the physical

and physiological quality of tomato seeds rather than washed directly using water.

Aside from providing good seeds, it is also needed to improve and optimize plant growth. In recent decades, physical techniques based on the application of magnetic fields are being developed in the agriculture sector. Studies showed that low magnetic field applications in agriculture can be used to improve the quality and quantity of the product. Numerous authors have reported the positive influence of the stationary magnetic field on the plant seeds.

Magnetic fields exposure of 125 mT and 250 mT for 12 hours increased the percentage of germination of lettuce (*Lactuca sativa* var. longifolia) seeds as well as root length, fresh weight and dry weight, and peroxidase enzyme activity of lettuce seeds (Mousavizadeh et al., 2013). Research on rice (*Oryza sativa* L.) seeds by Carbonell et al. (2000) showed that 150 mT and 250 mT magnetic fields increased germination. Giving a magnetic field affects the structure of cell membranes, so that permeability and ion transport which can affect metabolic pathways also increase (Iqbal et al., 2012).

Jedlicka et al. (2012) stated that a magnetic field could increase the germination speed, and vegetative and generative growth of tomato (Cakmak et al., 2010). Strong magnetic field induction as big as 0,2 mT could improve tomato plants' metabolism from the old seeds well so it could improve the vigor of seeds and vegetative growth of the tomato and also increase the chlorophyll and carbohydrates of the plants the same as the new seeds (Novitasari et al., 2019).

## Methods and Material

This research was done at Seed Technology Laboratory, Faculty of Agriculture, Universitas Padjadjaran from May to June 2022. Materials used in this experiment are tomato cv. Elisha, seedling media (cocopeat, NPL, fungicide), 2% HCl, 9% NaOCl, equates, and CD paper (blurred). Tools used are a solenoid magnetic field and its coil, trays, tweezers, pipettes, measuring cups, Petri dishes, knives, and strainers.

The experiment design used is Complete Random Design (CRD) factorial patern with two factors and three times replication. The first factor is seeds is the extraction methods, consisting of four treatments: E0 = control



(without extraction), E1 = soak by water 24 hours, E2 = HCl 2% 2 hours, and E3 = NaOCl 9% 15 minutes, and the second factor is magnetic field exposure: M0 = control (without exposure), M1 = 0,2 mT, M2 = 4 mT and M3 = 6 mT. The magnetic field was exposed for 7 minutes and 48 seconds (Rohma et al., 2013).

The seeds that were extracted according to the treatment were then exposed to the magnetic field in the amount of each treatment for 7 minutes and 48 seconds. Those seeds later on germinated with Top of The Paper Test (TP) method and used CD paper (blurred) on the petri dish and observed for 2 weeks to know the seed sprout's parameter.

Observing parameter consist of germination, maximum growth potential, growth rate, and simultaneous growth.

#### a) Germination Capacity (GC)

This observation was done twice, from the first-day count on day 5 and the last-day count on day 14. At the end of this period, the seedlings are categorized normal, abnormal, or dead or hard seeds. The observation was done by counting the normal seedling that meet the criteria for normal seedling and calculated by the formula as follows :

$$GC = \frac{\Sigma \text{ Normal Seedling}}{\Sigma \text{ Number of seed sown}} \times 100\%$$

#### b) Maximum growth potential (MGP)

Growth potential is counted based on the percentage of germinated seeds at the end of observation, which is day – after germination. Seeds germination was done with the same method as the germination capacity. The calculation formula is as follows:

$$MGP = \frac{\Sigma \text{ Grown seeds}}{\Sigma \text{ Planted seeds}} \times 100\%$$

#### c) Germination Speed Index (GSI)

Growth rate is calculated based on the percentage accumulation of normal seedling per etmal (24 hours) for the germination period which is until day 14 with the formula as follows:

$$GSI = \sum_0^{tn} \frac{N}{t}$$

Description:

t = Day of counting

N = Normal percentage every observation time

tn = Days of final count (day 14)

#### d) Uniformity Index (UI)

The calculation of uniformity index was done to the strong normal sprout on day 9, which is between the first day count (day 5) and last day count (day 14) after planting and stated in percent. The simultaneous growth used the equation formula as follows:

$$UI = \frac{\Sigma \text{ Normal seedling}}{\Sigma \text{ Planted sprouts}} \times 100\%$$

Data collected was then analyzed for the variance using SPSS software. If count showed significant effect, then it would be continued to Duncan Multiple Range Test (DMRT) on 5% real stage.

## Results and Discussion

The result of variance analysis on the impact of seeds extraction as a whole has a very significant effect on seed viability and vigor, while the effect of the magnetic field treatment only significantly affected the growth rate parameter and interaction between seeds extraction and magnetic field significantly affect on the germination capacity parameter.

**Table 1. Analysis of seeds extraction and magnetic field treatment to the tomato seed viability and vigor.**

Seed Quality Parameter	Treatment and Its Interaction		
	Extraction Technique (E)	Magnetic Field Exposure (M)	E X M
GC	**	ns	**
MGP	**	ns	ns
GSI	**	*	ns
UI	**	ns	ns

Description: GC (germination capacity), MGP (maximum growth potential), GSI (germination speed index), UI (uniformity index). \* (significantly different in 0.05 degree), \*\* (significantly different in 0.01 degree), ns (not significant).



Figure 1. Control



Figure 2. 24 hours fermentation

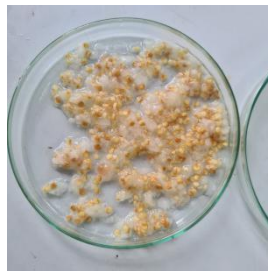


Figure 3. HCl 2% 2 hours



Figure 4. NaOCl 9% 15 minutes

**Tabel 2. The effect of extraction methods on germination capacity (GC) of tomato seeds**

Treatment	Germination Capacity (%)
Control (E0)	73.33 a
24 hours fermentation (E1)	93.00 b
HCl 2% 2 hours (E2)	89.67 b
NaOCl 9% 15 minutes (E3)	73.00 a

Description: Mean followed by the same letter on the same column showed no significant difference based on Duncan's Multiple Range test at 5% level.

The seeds extraction technique with fermenting tomato method for 24 hours showed the best germination percentage. This is because pulp on the seeds could be removed maximum such as shown in Figure 1. On the 2% HCl for 2 hours, there is seen a little pulp left and unclear color (Figure 2), and for control treatment and NaOCl it could be seen the pulp is still on the seeds (Figure 3 and 4). This is because acids are effective at cleaning the pulp attached to the seeds, where the longer the seeds are soaked, the more pulp is released from the seeds (Tarigan et al., 2018). Whereas in the control and NaOCl treatments it was seen that the pulp was still attached to the seeds (Figures 1 and 4). The slime will hinder the germination process, because it contains inhibitory substances.

The pulp would obstruct the germination process because it contains inhibitors. The red

color of the tomato contains lycopene pigment which is part of the carotenoids, and carotenoids are one of the raw materials for abscisic acid, a phytohormone inhibitor for plant physiological processes (Zoran et al., 2014).

Table 3 showed that tomato fermentation for 24 hours treatment is the treatment with highest percentage of maximum growth potential. It is suspected because when it is soaked in fermented water, seeds are imbibed well so it could improve seed viability and vigor. Good imbibition speed causes water needs for the seeds fulfilled so the seeds' metabolism process could go well (Juhanda et al., 2013).

The best magnetic field treatment for the maximum growth potential percentage parameter is 6 mT. Maximum growth potential percentage increases along with the improve of the amount of magnetic field exposure. It is because the magnetic field affects most of the cell membrane that could protect cells that are again surrounding by the membrane, and also it is no to induce seeds, respiration, and it's metabolism (Nurbaity et al., 2019).

According to the Table 4, the highest seed growth rate is also tomato fermentation seeds extraction for 24 hours treatment, it is because seeds germination power gave an impact on the seeds growth rate. Seeds that are clean of pulp will cause loss of germination inhibitory substance that exists in tomato pulp, so the less

pulp the faster the germination will be (Purba et al., 2018).

**Table 3. The effect of extraction techniques and magnetic field exposure treatments on maximum growth potential (MGP) of tomato seeds**

Treatment	Maximum Growth Potential (%)
<b>Seeds Extraction (E)</b>	
Control (E0)	79.33 a
24 hours fermentation (E1)	97.00 c
HCl 2% 2 hours (E2)	95.00 c
NaOCl 9% 15 minutes (E3)	88.00 b
<b>Magnetic Field (M)</b>	
Control (M0)	86.33 a
2 mT (M1)	89.00 ab
4 mT (M2)	90.00 ab
6 mT (M3)	94.00 b

Description: Mean followed by the same letter on the same column and factor showed no significant difference based on Duncan's Multiple Range test at 5% level.

**Table 4. The effect of extraction techniques and magnetic field exposure treatments on seed germination speed index (GSI) of tomato seeds**

Treatment	Germination Speed Index (GSI)
<b>Seeds Extraction (E)</b>	
Control (E0)	6.71 a
24 hours fermentation (E1)	15.68 c
HCl 2% 2 hours (E2)	12.95 b
NaOCl 9% 15 minutes (E3)	7.10 a
<b>Magnetic Field (M)</b>	
Control (M0)	9.15 a
2 mT (M1)	10.71 b
4 mT (M2)	11.52 b
6 mT (M3)	11.05 b

Description: Mean followed by the same letter on the same column and factor showed no significant difference based on Duncan's Multiple Range test at 5% level.

Ahamed et al. (2012) stated that paprika seeds exposed to the magnetic germinate one day faster than others that did not expose to the magnetic field. Seeds that are given a magnetic field also have the highest germination percentage rather to seeds that are only soaked in the water and seeds that are not exposed to the magnetic field. It is probably caused by the interaction between the magnetic field with the ion in plants' cell membranes that induces the change of osmosis pressure and ion

concentration on both sides of the cell membrane.

**Table 5. The effect of extraction technique on uniformity index (UI) of tomato seeds**

Treatment	Uniformity Index (%)
<b>Seeds Extraction (E)</b>	
Control (E0)	57.00 a
24 hours fermentation (E1)	94.33 b
HCl 2% 2 hours (E2)	89.00 b
NaOCl 9% 15 minutes (E3)	56.33 a

Description: Mean followed by the same letter on the same column showed no significant difference based on Duncan's Multiple Range test at 5% level.

From Table 5 it showed that that tomato soak by water for 24 hours is a treatment that has the highest percentage of simultaneous growth. High simultaneous growth indicates high seed vigor. It is in line with the previous parameter that showed tomato fermentation for 24 hours technique treatment has the highest seeds, vigor, and viability. On tomato fermentation for 24 hours technique treatment, the seeds will be soaked with the water from fermentation shed the seeds from the tomato seeds. The amount of time for fermentation is related to the pulp level (pulp) that still sticks on the seed coat surface. The pulp that remains on the seed coat could be the source of disease contamination on the seeds and could cause seeds vigor low (Daryanto & Yulianti, 2019). Tomato seeds that look clean from the pulp and have bright yellow color is a successful extraction process (Karavina et al., 2009).

## Conclusion

In general, seed extraction technique in form of fermentation for 24 hours and fermentation with 2% HCl could produce higher seed viability and vigor, but fermentation for 24 hours produced the best seed vigor. The exposure of the magnetic field only affected maximum growth potential with the best treatment in 6 mT. There was no interaction between both factors.

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Dewi TP · Hamdani JS · Mubarak S

## Response of growth and tuber seed production of G<sub>0</sub> potato (*Solanum tuberosum* L.) cv Medians in medium lands of Jatinangor to biochar composition and retardant type

**Abstract** Potatoes are the third most consumed food in Indonesia. The availability of high-quality potato tuber seeds is still low which affects potato production. Potato planting in the highlands is limited, so it needs to be expanded into medium lands. The modification that can increase the production of G<sub>0</sub> potato tuber seeds on medium lands is the use of biochar and retardant. The purpose of this research was to determine the composition of biochar and the type of retardant to increase the G<sub>0</sub> tuber seed yield of potato cv Medians in the medium lands of Jatinangor. The research was conducted from August 2022 to February 2023 at the Station Ciparanje, Jatinangor, Faculty of Agriculture, Universitas Padjadjaran. The experiment used Factorial Randomized Block Design (RBD). The first factor was the biochar compositions: b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), and b4 (80% compost + 10% biochar husk rice + 10% coconut shell biochar). The second factor was the retardant type: r1 (without retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadione-Ca). The experimental results showed that there was no interaction effect of biochar compositions and retardant type on the growth and yield of G<sub>0</sub> potato tuber seeds. Treatment of 80% compost + 20% coconut shell biochar suppressed plant height but increased the number of tubers. Treatment of 100 ppm paclobutrazol resulted in lower plant height but total chlorophyll content, percentage of stolon forming tubers, the number of tubers, and tuber weight per plant were higher compared to treatments without retardant and with 100 ppm prohexadione-Ca.

**Keywords:** Biochar · Medium lands · Potato · Retardant · Seed

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## Introduction

The potato (*Solanum tuberosum* L.) is one of the horticultural commodities with a high source of carbohydrate content, so it has the potential to be used in food diversification programs. Apart from being a source of carbohydrates, potatoes are rich in nutritional value, including minerals and vitamins, so they are included in the types of vegetables with high economic value, both as fresh and processed ingredients. This is due to potatoes being the third most widely consumed food in Indonesia, following rice and wheat (Statistics Indonesia, 2022b).

Statistics Indonesia (2022) states that potato production in Indonesia in 2021 has increased by 6.1% from 2020. However, potato production achieved in Indonesia in 2021 is still low because it can only meet Indonesia's potato consumption needs of 49.8%. The low production of potatoes in Indonesia is due to the low availability of good quality and quantity potato seeds (Hamdani et al., 2020). An essential factor for increasing potato production is the availability of high-quality seeds in sufficient quantities. Mulyono et al. (2017) stated that potato plants derived from G0 seeds had the highest increase in production.

Data from Statistics Indonesia (2022) shows that the harvested area of potato plants in Indonesia in 2021 decreased by 8% from 2020. One of the efforts to overcome this problem is the expansion of potato cultivation in medium lands, which is widely available in Indonesia. Differences in growing environmental conditions between the highlands and the medium lands are related to differences in temperature. According to Basu & Minhas (1991) in Prabaningrum et al. (2014), plants can experience high-temperature stress, which can increase gibberellin synthesis resulting in decreased photosynthate translocation to potatoes and increased translocation to leaves and stems.

In general, the soil in the medium lands is mostly Inceptisols. Inceptisols generally have less fertile soil properties, including slightly acidic soil pH, moderate C-organic levels, and low NPK nutrients (Yuniarti et al., 2020). However, it can still be improved with proper handling and technology. One way that can be done to increase the number and size of potato tuber seeds in the medium lands is by adding organic matter and biochar such as compost, rice husk charcoal, and coconut shell charcoal. In line with the results of Hamdani et al. (2017), the

application of ameliorants consisting of compost, coconut shell biochar, and dolomite can increase the number of tubers, and tuber weight per plant. According to the research results of Hamdani et al. (2019), the composition of the soil media, compost, rice husk charcoal, and cocopeat (1: 1: 1: 1) produced the highest values for the number of tubers and the weight of tubers per plant. However, the composition of the media is more expensive because there is more organic matter than the soil media. Thus, finding a cheaper media composition with a higher proportion of soil for G1 seed production in the medium lands is necessary.

Apart from planting media, another way that can be done is by applying growth regulatory substances, which can regulate the balance of plant growth. At the medium lands, elevated temperatures can lead to the accelerated synthesis of gibberellin at the tips of stems, young leaves, and roots, which function in the elongation and expansion of organs through cell growth which causes plants to become taller, slender, and chlorotic so that it has an impact on the potatoes formed few and small (Sumadi et al., 2015). Growth regulators that act as growth inhibitors include paclobutrazol and prohexadion-Ca (Hernawati et al., 2022).

From the problems in the medium lands, the composition of biochar and the correct type of retardant for planting potatoes in the medium lands has yet to be discovered. Therefore, this study aims to examine the effect of biochar composition and types of retardants on the growth and yield of potato cultivar medians in medium lands.

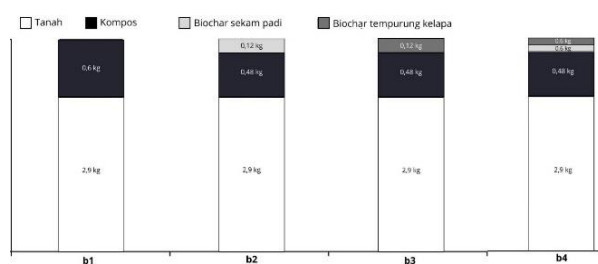
## Materials and Methods

The experiment was carried out from August 2022 to February 2023 at the Ciparanje Experimental Field, Faculty of Agriculture, Universitas Padjadjaran, with an altitude of  $\pm$  752 m above sea level.

The materials used in this experiment were potato seed G0 of the Medians cultivar, the soil of the order Inceptisol originating from the Ciparanje experimental field, compost, rice husk biochar, coconut shell biochar, Goldstar 250 SC (active ingredient Paclobutrazol 250 g/L), Prodex (active ingredient Prohexadione-Ca 15%), poly bag size 30 x 30 cm, plastic shade,



acetone 80%, label, urea fertilizer (46% N), SP-36 fertilizer (36% P<sub>2</sub>O<sub>5</sub>), fertilizer KCl (48% K<sub>2</sub>O), basamid 98 GR (98% Dazomet), insecticide Curracron 500 EC (500 g/L Profenofos), bactericidal Plantomycin (6.87% Streptomycin sulfate), fungicide Curzate 8/64 WP (64% Mankozeb) and the nematicide Furadan 3G (3% Carbofuran). The tools used in this experiment were hoes, weeding hoe, watering can, stake, measuring cup, hand sprayer, thermohygrometer, leaf porometer, spectrophotometer, test tubes, digital scales, oven, brown envelopes, and documentation equipment.



**Figure 1. Four different biochar composition treatments**

The experimental design used was a factorial randomized block design (RBD) consisting of two factors and three replications. The first factor is Biochar Composition (B), which consists of four levels, namely, b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar) and b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar). The second factor is Retardant Type (R), which consists of three levels, namely, r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Each experimental unit consisted of 5 plants, so there were 180 experimental plants placed according to the layout of the experiment.

Soil sterilized with basamid is given compost, rice husk biochar, and coconut shell on inceptisol soil following the proportions in Figure 1. The compost and biochar are weighed first with the dose used, which is 20 tonnes/ha (Rahayu, 2019). Then, the soil media was mixed according to the composition of the biochar treatment and put into polybags with the final weight of each treatment weighing 3.5 kg/polybag (polybag size 30 x 30 cm).

The application of paclobutrazol and

prohexadion-Ca according to the treatment with a concentration of 100 ppm was applied twice to the plants at 30 and 40 DAP. The volume of paclobutrazol and prohexadion-Ca used was based on the results of the previous calibration. The application of paclobutrazol and prohexadion-Ca is by spraying all the plant leaves using a sprayer.

Observations consisted of analysis of physical properties and pH of the planting media, plant height, total chlorophyll content, leaf carotenoid content, stomatal conductance, relative growth rate, tuber growth rate, plant dry weight, number of tuber and tuber weight per plant, and percentage of tuber seed class S, M and L. Experimental data on the effect of treatment were processed statistically using the F test at 5% significance level, while the difference in mean values between treatments using Duncan's Multiple Range Test analysis method at 5% significance level using SPSS data processing software version 23.0.

## Results and Discussion

**Analysis of the physical properties and pH of the planting media:** The results of the analysis of the physical properties and pH of the planting media in the experiment can be seen in Table 1. The 100% compost (b1) treatment had a higher density of 0.82 kg/L. The high density of the planting media indicates the denser the planting media, which means it is difficult to transmit water. These conditions can reduce porosity, causing a reduction in air space and water holding capacity of the planting media, which can inhibit root growth in controlling its capacity to absorb water and nutrients (Yulina & Ambarsari, 2021).

The mixture of planting media with a higher percentage of porosity and percentage of water holding capacity is 80% compost + 20% coconut shell biochar due to the addition of coconut shell biochar which has a larger surface area, the larger the pores of the biochar. This affects the ample space for air and water, which can increase the surface area of the pores of the planting media. The greater the space for air and water in the pores of the planting media, the planting media can absorb water to maintain its water content (Agviolita et al., 2021).

A mixture of 80% compost + 10% rice husk biochar + 10% coconut shell biochar has a higher percentage of air space, namely 12.40%. The

greater the percentage of air space in the planting media, the more oxygen is stored so that the respiration process of plant roots goes well.

The initial soil pH in the experiment was classified as neutral, namely 6.77, which was not under the pH needed by potato plants. The results of the laboratory analysis in Table 1 show that the pH of the treated biochar composition ranged from 5.87 to 6.48. The biochar composition is suitable for potato plants because the pH needed by potato plants is 5.0 – 6.5 (Diwa et al., 2015). The addition of compost and biochar in this experiment can lower the soil pH, making it suitable for the growth of potato plants.

**Plant Height, Leaf Area Index, Plant Dry Weight, and Root-Shoot Ratio.** The analysis results showed no interaction effect between the composition of biochar and the type of retardant on plant height, leaf area index, plant dry weight, and root-shoot ratio. However, independently the treatment of the biochar composition had a significant effect on plant height, while the retardant type had a significant effect on plant height, plant dry weight, and root-shoot ratio (Table 2).

Table 2 shows that a mixture of 80% compost + 10% rice husk biochar + 10% coconut shell biochar produced higher plant height than the other treatments but was not significantly different from the mixture of 80% compost + 20% rice husk biochar. The addition of rice husk biochar and coconut shell biochar to the planting media mixture increases soil porosity, water holding capacity, and air space so that it can improve the physical properties of the planting media and increase the availability of water and nutrients, which will make it easier for roots to absorb nutrients which will be transported to the vegetative parts of the plant so that vegetative growth such as plant height can be more optimal (Hamdani et al., 2020).

Treatment of 100 ppm paclobutrazol effectively suppressed plant height growth, plant dry weight, and root-shoot ratio. The application of paclobutrazol can inhibit the activity of gibberellin GA3 synthesis. GA inhibition causes plant cells to continue dividing, but new cells do not elongate, so branches with shorter book lengths are formed, resulting in shorter plant heights.

**Table 1. The results of the analysis of the physical properties and pH of the growing media**

Treatment	Density (kg/L)	Porosity (%)	Airspace (%)	Water Holding Capacity (%)	pH Media
b1	0.84	44.25	9.50	33.85	5.99
b2	0.72	48.00	10.40	38.50	5.87
b3	0.82	53.00	11.35	41.65	6.48
b4	0.75	49.00	12.40	36.60	5.97

Description: b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar) and b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar).

**Table 2. Effect of biochar composition and type of retardant on plant height, leaf area index, plant dry weight, and root-shoot ratio**

Treatment	Plant Height 60 DAP (cm)	Leaf Area Index	Plant Dry Weight (g)	Root-Shoot Ratio
<b>Biochar Composition (B)</b>				
b1	30.12 a	4.38	10.09	7.57
b2	35.37 b	4.58	9.71	8.54
b3	28.59 a	4.27	9.75	7.90
b4	37.84 b	4.72	9.15	8.02
<b>Retardant Type (R)</b>				
r1	37.08 b	4.78	12.06 b	9.76 b
r2	27.08 a	3.98	7.34 a	6.72 a
r3	34.78 b	4.71	9.62 ab	7.54 ab

Description: DAP is days after planting. b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar), r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Numbers marked with different letters represent significantly different according to Duncan's Multiple Range Test at the 5% level.

**Table 3. Effect of biochar composition and type of retardant on total chlorophyll content, leaf carotenoid content, and stomatal conductance**

Treatment	Total Chlorophyll Content (mg/l)	Leaf Carotenoid Content ( $\mu\text{mol/g}$ )	Stomatal Conductance ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )
<b>Biochar Composition (B)</b>			
b1	31.36	1391.57	184.84
b2	29.28	1336.90	178.39
b3	31.46	1295.45	201.33
b4	30.39	1441.04	199.35
<b>Retardant Type (R)</b>			
r1	28.61 a	1093.35 a	155.94 a
r2	33.23 b	1484.45 b	206.85 b
r3	30.03 a	1520.92 b	210.15 b

Description: DAP is days after planting. b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar), r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Numbers marked with different letters represent significantly different according to Duncan's Multiple Range Test at the 5% level.

When observing the dry weight of plants and the ratio of root-shoots, the plants entered the age of 60 DAP, so the potato plants had entered a phase in the formation of potatoes towards the old phase. In this phase, more photosynthate in the leaves is translocated to fill the potato. This is supported by Hamdani et al. (2018), that the application of paclobutrazol at 45 DAP enters the end of the plant growth phase, so the photosynthate results in the leaves are distributed for filling potatoes.

**Total Chlorophyll Content, Leaf Carotenoid Content, and Stomatal Conductance.** The analysis results showed no interaction effect between biochar composition and retardant type on total chlorophyll content, leaf carotenoid content, and stomatal conductance. However, the treatment of biochar composition had no significant effect on total chlorophyll content, leaf carotenoid content, and stomatal conductance, while the type of retardant had a significant effect on total chlorophyll content, leaf carotenoid content, and stomatal conductance (Table 3).

One of the factors that influence the formation of chlorophyll is water availability. The chlorophyll content, which was not significantly different, presumably indicated that all the treatments given were able to provide sufficient water for plants to carry out chlorophyll synthesis. The low availability of water can inhibit the absorption of nutrients because one of the functions of water is as a solvent for nutrients, especially nitrogen and

magnesium, which play an essential role in the synthesis of chlorophyll. According to Zhao et al. (2015) in Yuniati (2020), increased stomatal conductance correlates with high water uptake by roots. The high stomatal conductance values show that plants are in good condition to carry out photosynthesis (Soleh et al., 2020).

Treatment of 100 ppm paclobutrazol resulted in the highest total chlorophyll content, carotenoid content, and stomatal conductance compared to the control treatment. Paclobutrazol can stimulate the synthesis of cytokinin hormones, which increases chloroplast differentiation and chlorophyll synthesis (Xia et al., 2018 in Yuniati, 2020) and encourages stomata opening (Pal et al., 2016 in Yuniati, 2020). Chlorophyll requires carotenoid accessory pigments, which can increase the binding of photons from sunlight in the light reaction of photosynthesis. Carotenoids can expand the range of light absorption by absorbing the spectrum of sunlight in the green-blue section, then passing it on to chlorophyll to help in photosynthesis (Hashimoto et al., 2016). Likewise, opening larger stomata and increasing stomatal density can increase  $\text{CO}_2$  concentration in chloroplasts which is a factor in increasing stomatal conductance (Xia et al., 2018 in Yuniati, 2020).

**Relative Growth Rate, Tuber Growth Rate, Number of Stolons, and percentage of stolons forming tubers.** The analysis results showed no interaction effect between the composition of biochar and the type of retardant

on the relative growth rate, the tuber growth rate, the number of stolons, and the percentage of stolons forming tubers. However, independently the treatment of biochar composition had a significant effect on the growth rate of potatoes, while the retardant type had a significant effect on the growth rate of potatoes, the number of stolons, and the percentage of stolons forming tubers (Table 4).

Table 4 shows that a mixture of 80% compost + 20% rice husk biochar produced the highest tuber growth rate compared to the 100% compost treatment but was not significantly different from the mixture of 80% compost + 20% coconut shell biochar and 80% compost + 10% rice husk biochar + 10% coconut shell biochar. The addition of rice husk biochar and coconut shell biochar can create conditions for planting media with a reasonably fine structure,

good drainage without a waterproof layer, and the availability of sufficient nutrients and water for optimal plant growth, especially in the process of photosynthesis so that the resulting photosynthate is channeled and stored as food reserves in potatoes.

Treatment of 100 ppm paclobutrazol produced the lower than number of stolons. However, the growth rate of potatoes and the percentage of stolons forming tubers were higher than the treatment without retardant but not significantly different from the 100 ppm prohexadion-Ca treatment. It is suspected that earlier retardant treatment applied two times (30 and 40 DAP) was able to increase the effectiveness and work of retardants in inhibiting gibberellin synthesis to inhibit stolon elongation. However, there was an increase in the percentage of stolons forming tubers.

**Table 4. Effect of biochar composition and type of retardant on relative growth rate, tuber growth rate, number of stolons and percentage of stolons forming tubers**

Treatment	Relative Growth Rate	Tuber Growth Rate	Numbers of Stolon	Tuber Formed of Stolon (%)
<b>Biochar Composition (B)</b>				
b1	0.021	0.46 a	5.00	70.81
b2	0.029	1.05 b	5.88	55.59
b3	0.031	0.55 ab	4.44	47.96
b4	0.053	0.63 ab	5.22	49.88
<b>Retardant Type (R)</b>				
r1	0.020	0.22 a	6.67 b	38.37 a
r2	0.047	0.91 b	4.00 a	72.27 b
r3	0.034	0.89 b	4.75 ab	57.54 ab

Description: DAP is days after planting. b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar), r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Numbers marked with different letters represent significantly different according to Duncan's Multiple Range Test at the 5% level.

**Table 5. Effect of biochar composition and type of retardant on the number of tubers, tuber weight and percentage of tuber seed quality class**

Treatment	Number of Tubers (knol)	Tuber Weight (g per plant)	Percentage of Tuber Seed Quality Class		
			S (%)	M (%)	L (%)
Biochar Composition (B)					
b1	5.18 a	51.58 a	95.00	5.00	0.00
b2	6.18 b	63.44 b	94.05	5.95	0.00
b3	6.29 b	58.74 ab	96.97	3.03	0.00
b4	6.04 ab	56.84 ab	91.97	6.79	0.00
Retardant Type (R)					
r1	5.22 a	45.65 a	98.33	1.67	0.00
r2	6.38 b	61.54 b	91.79	8.20	0.00
r3	6.17 b	53.79 ab	93.36	5.71	0.00

Description: DAP is days after planting. b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar), r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Numbers marked with different letters represent significantly different according to Duncan's Multiple Range Test at the 5% level.

**Table 6. Effect of biochar composition and type of retardant on shoot emergence time, seed germination percentage, and seeds weight loss**

Treatment	Shoot Emergence Time (Day)	Seed Germination Percentage	Seeds Weight Loss (%)
<b>Biochar Composition (B)</b>			
b1	62.22 b	44.44	2.95
b2	38.88 a	51.11	3.40
b3	43.33 a	31.11	4.81
b4	45.55 a	53.33	3.33
<b>Retardant Type (R)</b>			
r1	40.00	33.33	4.16
r2	45.83	53.33	3.62
r3	50.83	48.33	3.09

Description: DAP is days after planting. b1 (100% compost), b2 (80% compost + 20% rice husk biochar), b3 (80% compost + 20% coconut shell biochar), b4 (80% compost + 10% rice husk biochar + 10% coconut shell biochar), r1 (Without Retardant), r2 (100 ppm paclobutrazol), and r3 (100 ppm prohexadion-Ca). Numbers marked with different letters represent significantly different according to Duncan's Multiple Range Test at the 5% level.

In line with the opinion of Hamdani et al. (2018) that the timing of paclobutrazol application is essential at the beginning of the tuber formation phase (30 DAP) because it can cause a decrease in shoot growth but can increase tuber formation due to increased assimilate which is diverted to the process of tuber formation. Observations made at 50 and 60 DAP entered the potato filling phase so that the growth rate would continue to increase.

**Number of Tubers, Tuber Weight, and Percentage of Tuber Seed Quality Class.** The analysis results showed no interaction effect between the biochar composition and the type of retardant on the number of tubers, tuber weight and percentage of tuber seed quality class. However, the treatment of the biochar composition had a significant effect on the number of tubers and the tuber weight, while the retardant type had a significant effect on the number of tubers and the tuber weight (Table 5).

A mixture of 80% compost + 20% coconut shell biochar produced numbers of tuber and tuber weight higher than the 100% compost treatment but was not significantly different from the other treatments. The high rate of tuber growth rate influences this because the addition of coconut shell biochar and rice husk biochar has a high percentage of porosity and water-holding capacity, so the water and air conditioning of the planting media is good for the growth and development of potatoes. In line with Rahayu's research (2019), the treatment of 80% compost + 20% coconut shell biochar produced the highest amount of tuber plant compared to the 100% compost treatment.

Rafindo et al. (2022) stated that many pore spaces can increase the absorption of water and nutrients so that the photosynthetic capacity of plants increases. The photosynthetic capacity of plants is a factor in tuber formation.

The 100 ppm paclobutrazol treatment produced the highest number and weight of potatoes compared to the non-retardant treatment but was not significantly different from the 100 ppm prohexadion-Ca treatment. This is related to the concentration of the retardant given, which is 100 ppm, and the frequency of giving the retardant twice at 30 and 40 DAP. Based on the research results of Hamdani et al. (2018), the application of paclobutrazol at a concentration of 100 ppm can increase the weight of potatoes per plant compared to no application of paclobutrazol. Likewise, with the results of Aulia's research (2020), applying 100 ppm paclobutrazol and applying 100 ppm prohexadione-Ca at 40 DAP can increase the weight of potatoes. The retardant application time given at 30 and 40 DAP caused vegetative growth to be inhibited, and optimal assimilate transfer occurred to support the formation of potatoes because, at this age, the plant was entering the phase of potato formation.

The percentage value of class S G1 seed potatoes in the treatment of biochar composition and retardant types has a higher percentage than M and L classes. The high percentage of class S G1 seed potatoes in potato planting with the aim of seed production will follow market demand. In line with the results of research by Adiyoga et al. (2014) that respondent farmers prefer tubers

measuring 30 - 40 g, which is included in class S G2 seed potatoes because it has a smaller weight and size, so it is easy to store and distribute.

**Time of Shoots Emergence, Percentage of Seeds Sprouting, and Weight Loss of Seeds.** The analysis results showed no interaction effect between the biochar composition and the type of retardant at the time of shoot emergence, the percentage of seeds sprouting, and the weight loss of seeds. However, independently the treatment of biochar composition had a significant effect on the time of emergence of shoots, while the retardant type had no significant effect on the time of emergence of shoots, percentage of seeds sprouting, and weight loss of seeds (Table 6).

The time the shoots appear is related to the breaking of the seed dormancy period, marked by the emergence of shoots. The potato dormancy period reaches three months to more than five months. Factors that affect the duration of dormancy include the place of planting during the growth period and the age of the potato in the field (Sahat et al., 1989 in Nuraini et al., 2016). The addition of biochar resulted in a faster shoot emergence than without the addition of biochar. This is related to the potato's higher weight value produced by adding biochar because there is a potential for food reserves contained in the potato, which is the result of photosynthesis, thereby accelerating the growth of new shoots. However, on observation 70 days after harvest, the percentage of germinated seeds was 31.1 -53.33%. This is presumably because, at that time, the potato was still dormant. At the time of observation, some of the tuber began to wrinkle on the skin of the tuber. This was influenced by the high weight loss of the tuber, which could result in changes in the physiological quality of the seeds. Wiersema (1989) in Purnomo et al. (2017) stated that water loss from potato tubers would be more significant if the potatoes had sprouted.

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## Conclusion

From the experimental results and discussion, it can be concluded as follow.

1. There was no interaction between biochar composition and retardant type on the growth and yield of G0 potato seed cultivar Medians in the Jatinangor medium plains.
2. Providing 80% compost + 20% coconut shell

biochar can reduce plant height and increase the number of tubers per plant.

3. Application of 100 ppm paclobutrazol resulted in lower plant height but produced the highest total chlorophyll content, number of tubers, and tuber weight per plant.

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## Response of growth, yield, and seeds quality of G<sub>0</sub> potato on different potassium fertilizer doses and retardant concentrations in Jatinangor medium land

**Abstract.** This study aims to determine the best interaction of potassium (K) fertilizer dose and retardant concentration on the growth, yield, and seed quality of G<sub>0</sub> potato grown in the medium land of Jatinangor. The experiment was conducted at Screen House Station Ciparanje, Jatinangor, Faculty of Agriculture Universitas Padjadjaran from November 2022 until January 2023 at an elevation of ± 685 meters above sea level. The experimental design used was the Factorial Randomized Block Design (RBD). The first factor was the K fertilizer dose including 50%, 100%, and 150%. The second was the retardant concentration including without retardant, 100 ppm paclobutrazol, 100 ppm prohexadione-Ca, and 150 ppm prohexadione-Ca. The experimental results showed that there was an interaction effect between the K dose and retardant concentration. The interaction effect of 150% K and 100 ppm prohexadione-Ca showed the highest percentage of L class seed tuber and the lowest tuber weight loss. Independently, 150% K improved growth rate, number of tubers (6.67 knol/plant), and weight of tubers (73.33 g/plant). Independently, 100 ppm paclobutrazol concentration increased the chlorophyll content index. 150 ppm prohexadione-Ca concentration suppressed leaf area and plant height but increased stomatal conductance, fast emergence shoot time, and shoot length. 100 ppm prohexadione-Ca concentration produced the highest number of tubers and the weight of tubers, i.e., 6.78 knol/plant and 74.33 g/plant, respectively.

**Keywords:** Paclobutrazol · Potassium · Potato Seed · Prohexadione-Ca

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## Introduction

Potato (*Solanum tuberosum* L.) is one of the horticultural crops with tuber as its commercial part. Indonesia is one of the countries that has developed potato commodities, both for direct consumption as a vegetable and for industry. The need for potatoes is increasing year by year, along with the increasing population of Indonesia. Based on the Statistics Indonesia (2020) potato production in Indonesia in 2020 reached 1.28 million tonnes which shows a decrease of 2.42% from 2019.

Efforts to increase the production of potatoes are intensification of cultivation techniques. Improving the cultivation technique can be in the use of high-quality seeds which include genetic quality, physical quality, and physiological quality, because seed is the key factor in determining the final yield obtained (Hamdani et al., 2021). However, the availability of high quality potato seeds in Indonesia is still limited. The availability of high quality potato seeds in 2015 only met 31% of the national need for potato seeds or 125,000 tons (Ranti et al., 2017). According to Hamdani et al. (2020), the low availability of quality potato seeds forces some farmers to re-use seeds produced from previous plantings.

The potato cultivar (cv.) Medians can adapt well in medium plains (Supriatna et al., 2018). This potato cultivar has high productivity, reaching 31.9 tonnes ha<sup>-1</sup> (Vegetable Crops Research Institute, 2018). The Medians cultivar is the result of selection from the progeny of a cross between Atlantic as one of its parents, so the characteristics of the Medians cultivar are similar to Atlantic but its production is higher. Median potatoes are a type of processed potato with a high yield, making it suitable for the needs of the potato chip manufacturing industry. It is hoped that the development of the potato cv. Median can fulfil some of the needs of the increasingly increasing yield processing industry. The need for potatoes for large industries can reach 30 tonnes/day.

The main problem in G<sub>0</sub> seed production from cuttings is the low formation of tubers from stolon, so the number of tubers produced per plant is small. One effort to solve the problem of increasing the number of potato produced is by applying potassium fertilizer. Potassium (K) fertilization is expected to increase the growth and production of both the

quantity and quality of the potato tubers produced. Zelelew et al. (2016) reported that potassium fertilization greatly affected the growth and production of potatoes. The results of Gunadi's research (2007) showed that the application of 50 kg ha<sup>-1</sup> of potassium fertilizer could produce a marketable percentage of potato tubers of 81.8%. The results of the research by Sitepu et al. (2011) stated that the application of KCl fertilizer 20 g m<sup>-2</sup> resulted in the largest tuber diameter, fresh tuber weight per plant and dry yam weight per plant which were 3.72 cm, 186.9 g and 56.1 g sequentially.

Potassium fertilizer application is expected to affect increasing yield growth and optimal quality of G<sub>0</sub> potato seed yields. Potato plants can grow optimally if the necessary nutrients can be fulfilled. K fertilizer contains the element K which is one of the macronutrients needed by potato plants in the process of forming and filling potato because K functions to stimulate assimilate translocation from the leaves to the potato parts. In addition, potassium in potato plants will be needed for the accumulation and translocation of the carbonate formed by plants from the results of photosynthesis and plant physiological responses. Potassium also functions as a catalyst for protein formation, sugar translocation, increasing water use efficiency, expanding root growth, strengthening plant tissues and organs so they don't fall off easily, increasing plant resistance to pest attacks, and increasing the quality and quantity of tuber (Martias, 2011).

In addition, using K fertilizer can overcome potato cultivation in medium plains which have high temperatures reaching more than 20°C. High temperatures in potato plants increase the synthesis of gibberellins, causing the translocation of photosynthetic products to the top of the plant and reducing the translocation of photosynthetic products to tuber. High temperatures cause the synthesis of gibberellin to take place more quickly at the tips of stems, young leaves and roots. If gibberellin synthesis is not inhibited, it can result in plant growth being focused on the top of the plant continuously and inhibiting the formation of tuber. Therefore, it is necessary to apply plant growth inhibitors to balance the growth of potato plants. Giving growth inhibitors can be done as an effort to increase the yield and quality of G<sub>0</sub> potato seeds from cuttings in the hope that they can regulate the balance of

growth and yield. One of the growth regulators that act as inhibitors are retardants, namely paclobutrazol and prohexadione-Ca.

Paclobutrazol is a growth regulator that inhibits vegetative growth and inhibits gibberellin biosynthesis which functions in the process of elongating plant cells and tissues (Masniawati, 2016). According to Hamdani et al. (2016) potato plants treated with inhibitory hormones are intended to speed up the filling phase of potato tubers and can focus energy on the formation of tuber. The use of paclobutrazol can regulate plant growth patterns by maintaining a balance between generative and vegetative growth, so that competition in the utilization of photosynthates-producing locations or sources for vegetative growth and tuber formation can be suppressed. Giving paclobutrazol will affect the decrease in plant height, increase in chlorophyll content, fresh weight of tuber, yield per hectare, and percentage of the quality class of tuber (Hamdani et al., 2021).

Apart from paclobutrazol, another inhibitor for the body is prohexadione-Ca. Prohexadione-Ca is a growth inhibitor that has the same physiological properties as paclobutrazol but does not leave a long-term residue. According to Adiel et al. (2011), Prohexadione-Ca is a growth inhibitor with a short residual effect lasting only a few weeks. Thus, the use of Prohexadione-ca can inhibit vegetative plant growth and induce the growth of potato stolons and tubers without causing any risk to the soil. The prohexadione-Ca chemical substance is a type of retardant substance whose way of action is similar to other anti-gibberellin retardants such as paclobutrazol, coumarin, chlormequat chloride (CCC) which inhibits gibberellin biosynthetic activity in plants (Kofidis et al., 2008).

The effectiveness of using paclobutrazol and prohexadione-Ca is influenced by the concentration and time of application, the time of application is related to the conditions of the plant growth stage. The concentration of retardant substances needs to be given precisely to each plant because each plant will show a different response depending on the genotype and physiological conditions of the plant (Lestari, 2011). Giving growth regulators with excessive concentrations will disrupt cell functions as well as if the use of concentrations that are less than the optimal concentration then the use of these substances becomes invisible

(Kamillia et al., 2019). Another thing to note is the growing stage of the plant. The use of growth regulators will not respond if they are not given during the sensitive period of the plant. The sensitive period is the time of application according to the plant growth stage (Hamdani et al., 2019). Prohexadione-Ca and paclobutrazol are inhibitors of gibberellin synthesis, so to increase potato yields both must be applied at the beginning of the tuber formation phase, which is between 30-50 DAP.

In this research, the application of K fertilizer combined with retardants, namely paclobutrazol, and prohexadione-Ca, is expected to have a better impact on increasing the number and weight of potato seeds planted in medium plains. This refers to the results of Yuniarti's research (2017), which showed that 150 kg/ha K fertilizer treatment with a 100 ppm paclobutrazol concentration could increase the weight of the potato plants, the chlorophyll content and the starch content of the potato. The research results of Wijana et al. (2015) showed that there was an interaction between the concentration of paclobutrazol and the dose of K fertilizer which had a significant effect on the number of leaves and the yield of shallots. Furthermore, according to Conover (1994), plants given retardants such as paclobutrazol usually require less water and fertilizer than those without paclobutrazol treatment. however, there is still limited studies on K fertilizer and retardant application for seed potato production. Therefore, this study aims to determine the best interaction of potassium (K) fertilizer dose and retardant concentration on the growth, yield, and seed quality of  $G_0$  potato grown in the medium land of Jatinangor.

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## Materials and Methods

The tools used in this experiment were hoes, calipers, hammers, digital scales, cutters, documentation equipment, and stationery. The materials used in this experiment were seed cuttings of the Medians potato aged 14 days after acclimatization from PT. Horti Agro Macro. The planting medium uses the soil of the order Inceptisol originating from the Ciparanje experimental, polybag, mica plastic, stakes, silver-black plastic mulch, rope plastic, snack box, compost, husk charcoal, cocopeat, retardant substance Prodex® containing the active

ingredient Prohexadione-Calcium 15% WP and Golstar containing the active ingredient Paclobutrazol 250 SC, Fungicide Plantomycin (Streptomycin Sulfate 6.87%), Antracol fungicide (Propineb 70%), Basamid 98 GR (Dazomet 98%) for media sterilization, Curacron insecticide (Profenofos 500 g/L), urea fertilizer (46% N), SP-36 (36% P<sub>2</sub>O<sub>5</sub>) and KCl (60% K<sub>2</sub>O).

## Results and Discussion

**Chlorophyll Content Index (CCI) and Stomatal Conductance.** The results of the statistical analysis showed that there was no interaction between the doses of K fertilizer and the retardant concentration on the index of chlorophyll content and stomatal conductance. The independent effect of the treatment of K fertilizer dose and retardant concentration on the index of chlorophyll content and stomatal conductance is presented in Table 1.

The treatment of retardant concentrations had a significantly different effect on the index of chlorophyll content. The concentration of 100 ppm paclobutrazol showed the highest chlorophyll content index, namely 52.90 but not significantly different from 150 ppm prohexadione-Ca. In line with research conducted by Nuraini et al. (2021); Hernawati et al. (2022) that the treatment of paclobutrazol increased chlorophyll content. This is presumably because paclobutrazol can increase the synthesis of chlorophyll in the leaves (Tekalign et al., 2005). Giving paclobutrazol could affect the increase in chlorophyll content, decrease in plant height, fresh weight of tuber, yield per hectare, and percentage of the quality class of tuber (Hamdani et al., 2021).

The physiological role of the retardant paclobutrazol is to suppress stem elongation,

thicken the stem, and encourage the formation of pigments (chlorophyll, xanthophyll, anthocyanin) which can produce a darker green leaf color with high chlorophyll content. The chlorophyll content is one of the factors that affect plant metabolism through the process of photosynthesis. According to Wieland and Wampe (1985) in Sambeka et al. (2012), the application of paclobutrazol increases the chlorophyll content of leaves so that photosynthetic activity can run well and inhibition of shoots stimulates photosynthetic results to be used for the formation of carbohydrates. The higher the rate of photosynthesis in plants, the higher the growth and productivity of plants. The leaf chlorophyll content is one of the main factors affecting the photosynthetic capacity of potato plants (Kantikowati et al., 2019).

Dosing of K fertilizer also did not have a significantly different effect on stomatal conductance, but retardant had a significant effect on stomatal conductance. Treatment with a concentration of 150 ppm prohexadione-Ca showed higher stomatal conductance, but was not significantly different from the treatment with a concentration of 100 ppm paclobutrazol and 100 ppm prohexadione-Ca. The concentration of 150 ppm prohexadione-Ca gives a high stomatal conductance response, high stomatal conductance indicates the plant is in good condition for photosynthesis or metabolism. Stomata are a tool for the passage of CO<sub>2</sub> and H<sub>2</sub>O gases from outside the plant into the plant so that metabolic processes go hand in hand with the opening and closing of stomata, if metabolism is hampered then the conductance of the stomata could decrease and even stop (Soleh et al., 2017). Several factors affect stomatal conductance including phytohormones, light, drought stress, carbon dioxide, and signals originating from microbes (Ye et al., 2020).

**Table 1. Effect of different K fertilizer doses and retardant concentrations on chlorophyll content index and stomatal conductance of G<sub>0</sub> potato**

Treatment	Chlorophyll Content Index (CCI)	Stomatal conductance (μmol H <sub>2</sub> O/m <sup>2</sup> s)
d <sub>1</sub> : 50% dose of K (50 kg KCl/ha)	29.26 a	47.84 a
d <sub>2</sub> : 100% dose of K (100 kg KCl/ha)	32.89 a	41.39 a
d <sub>3</sub> : 150% dose of K (150 kg KCl/ha)	32.10 a	46.25 a
r <sub>1</sub> : without retardan	46.61 a	49.64 a
r <sub>2</sub> : 100 ppm paclobutrazol	52.90 b	53.93 ab
r <sub>3</sub> : 100 ppm prohexadione-Ca	32.49 a	65.71 ab
r <sub>4</sub> : 150 ppm prohexadione-Ca	35.56 ab	71.59 b

Note : Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level.

**Table 2. Effect of different K fertilizer doses and retardant concentrations on relative growth rate and potato growth rate of G<sub>0</sub> potato**

Treatment	Relative Growth Rate (g g <sup>-1</sup> day <sup>-1</sup> )	Tuber Growth Rate (g plant <sup>-1</sup> day <sup>-1</sup> )
d <sub>1</sub> : 50% dose of K (50 kg KCl/ha)	0.0260 a	0.0924 a
d <sub>2</sub> : 100% dose of K (100 kg KCl/ha)	0.0150 a	0.127 ab
d <sub>3</sub> : 150% dose of K (150 kg KCl/ha)	0.0156 a	0.232 b
r <sub>1</sub> : without retardant	0.0379 a	0.187 a
r <sub>2</sub> : 100 ppm paclobutrazol	0.0129 a	0.162 a
r <sub>3</sub> : 100 ppm prohexadione-Ca	0.0287 a	0.272 b
r <sub>4</sub> : 150 ppm prohexadione-Ca	0.0212 a	0.181 a

Note : Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level.

**Relative Growth Rate and Tuber Growth Rate.** The results of the analysis can be seen in Table 2. That the application of K fertilizer doses and retardant concentrations did not have a significantly different effect on the relative growth rate (RGR). RGR illustrates an increase in plant dry weight at certain time intervals (Nurmuliana & Akib, 2019). The growth rate is relatively closely related to plant dry weight, which is then divided per unit of time to see the increase in plant dry weight. There was no significant difference in the treatment of the relative growth rate of plants, meaning that the growth rate of all plants was almost uniform at the age of 50-60 DAP. That time the plants entered the advanced vegetative growth phase which caused all treatments to have the same rate so that the growth rate was relatively not different.

Furthermore, from Table 2. Data can be seen that the dose of K fertilizer and the concentration of the retardant had a significantly different effects on the rate of growth of tuber. The rate of growth of tuber is a parameter that describes the speed of assimilate accumulation in the tuber. The highest growth rate of potato was in the treatment of 150% dose of K (is 0.232 g plant<sup>-1</sup> day<sup>-1</sup>). The element K is directly involved in plant physiological processes, namely it plays a role in enzyme activation, stimulates assimilation and assimilates transport. Potassium triggers the translocation of carbohydrates from leaves to carbohydrate-storing plant organs. According to Pahlevi et al. (2016), potassium plays a role in the assimilate translocation process from the source (source) to the storage (yam), Helal & Abdelhady (2015) added that potassium can increase tuber yield a plant.

The 100 ppm prohexadione-Ca treatment gave a significantly different effect on the growth rate of tuber, namely 0.272 g plant<sup>-1</sup> day<sup>-1</sup>. TGR is greatly affected by environmental factors such as temperature. High temperatures can cause low levels of photosynthesis in providing assimilation for all plant growth and can reduce the distribution of carbohydrates to tuber. According to Azima et al. (2017), the formation of potato is also strongly influenced by the photosynthetic capacity of plants, one of the results of plant photosynthesis could be translocated to the stolon section for initiation of potato. Giving prohexadione-Ca could focus the results of photosynthesis on the tuber. The larger the photosynthate partition results, the greater the assimilation that can be transferred to the tuber. High temperatures can also shorten the filling time of tuber, because high temperatures stimulate the synthesis of gibberellin which inhibits the initiation of tuber. Prohexadione-Ca is a compound that can cause decreased growth by blocking gibberellin biosynthesis, thereby inhibiting vegetative growth (Adiel et al., 2011).

**Tuber Number and Tuber Weight per Plant.** The results of the analysis of variance showed that there was no interaction effect of K fertilizer dosing and concentration inhibition on the number of tuber and the weight of the tuber. Independently application of K fertilizer dose and retardant concentration showed a significant effect on the number of tuber and the weight of G<sub>0</sub> potato tubers from cuttings. The independent effect on the number of tuber and the weight of tuber is presented in Table 3



**Table 3. Effect of different K fertilizer doses and retardant concentrations on number of tuber and weight of tuber of G<sub>0</sub> potato**

Treatment	Number of Tuber Per Plant (knol)	Weight of Tuber Per Plant (g)
d <sub>1</sub> : 50% dose of K (50 kg KCl/ha)	5.42 a	58.83 a
d <sub>2</sub> : 100% dose of K (100 kg KCl/ha)	5.17 a	55.42 a
d <sub>3</sub> : 150% dose of K (150 kg KCl/ha)	6.67 b	73.33 b
r <sub>1</sub> : without retardant	4.44 a	49.78 a
r <sub>2</sub> : 100 ppm paclobutrazol	6.56 bc	69.67 bc
r <sub>3</sub> : 100 ppm prohexadione-Ca	6.78 c	74.33 c
r <sub>4</sub> : 150 ppm prohexadione-Ca	5.22 ab	56.33 ab

Note : Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level.

The dose of K fertilizer and the concentration of the retardant had a significantly different effects on the number of potato tubers. The treatment of 150% dose K showed the number of tubers per plant was more when compared to the 50% dose K and 100% dose K treatments. This was due to an increase in the dose of K fertilizer which could stimulate the formation of tuber, so that the number of tuber produced was optimal (Osman, 1996). K plays a role in the process of photosynthesis because K is an activator of various essential enzymes. The formation of tuber is strongly influenced by the accumulation of photosynthate from the process of photosynthesis. Some of the results of photosynthesis could be translocated to the roots to initiate the formation of tuber (Gutomo et al., 2015). So that in the process of forming potato tubers, K is needed which is quite high. This is in accordance with the results of the experiments carried out, namely the 150% dose of K (d<sub>3</sub>) treatment was able to produce more potato tubers compared to other K fertilizer dose treatments.

The treatment of retardant concentrations had a significantly different effect on the number of tuber per plant. The 100 ppm prohexadione-Ca treatment showed a high amount of tuber, but it was not different from the 100 ppm paclobutrazol treatment. Giving prohexadione-Ca could inhibit the endogenous gibberellin content so that the stolons stop elongating and begin to enlarge at the ends of the stolons to form and produce tuber in large quantities. An increase in gibberellins in potato plants grown in unsuitable conditions, namely high temperatures such as in medium plains could certainly lead to an increase in gibberellin synthesis in stolons so that a large number of

stolons grow. The temperature in the field is quite high, which causes an increase in gibberellin synthesis in stolons so that the number of stolons increases (Struik, 2007).

A large number of stolons have the opportunity to become tuber if there is a stimulus for potato initiation. Paclobutrazol and prohexadione-Ca are thought to play a major role in the differentiation of stolons into tuber, so that more tuber are formed. The use of retardants accelerates the occurrence of tuberization. According to Sakia et al. (2013), giving retardants can reduce gibberellin activity so that it can inhibit the vegetative phase such as stolon elongation so that it can focus the flow of photosynthate for the formation and enlargement of tuber.

The dose of K fertilizer had a significantly different effect on the weight of potato tubers per plant. 150% of the given K dose resulted in the highest G<sub>0</sub> potato weight of 73.33 g per plant. It is suspected that in the treatment of 150% dose of K, the nutrient K available and absorbed by plants was more than in the other treatments. This is in accordance with the results of the analysis of K content that the treatment of 150% dose of K showed the highest K nutrient content, namely 0.09%. The availability of high K nutrients can help enlarge potato tubers and be able to translocate the assimilated results from photosynthesis to maximize tuber formation because K fertilizer is needed by plants in the formation of carbohydrates in tuber, increases leaf resistance and water absorption so as to prevent wilting and can enlarge potato size. According to Hanafiah (2010), K can make the photosynthesis process and photosynthate distribution running smoothly. Sutrisna et al. (2003) stated that the balance of K nutrients in

the soil plays an important role in the synthesis of carbohydrates and proteins so it greatly helps grow tuber.

Giving retardant concentrations in the 100 ppm prohexadione-Ca treatment had a significant effect on producing a higher tuber weight of 74.33 g per plant compared to other treatments. Prohexadione-Ca with a concentration of 100 ppm was able to suppress the synthesis of gibberellins so that the growing stolons stopped elongating and then formed tubers. The high or low weight of the tuber formed depends on the amount of assimilates that can be produced by the plant. The more assimilates produced by a plant, the higher the weight of the tuber and vice versa. This is consistent with the observation of the rate of growth of potato that the 100 ppm prohexadione-Ca treatment showed the highest growth rate of potato, namely  $0.272 \text{ g plant}^{-1} \text{ day}^{-1}$ .

Under these conditions, according to (Sutater et al., 1993) the number of photosynthates that flowed and stored as food reserves determine the weight of tuber because tuber are a food reserve resulting from photosynthesis. Increasing the formation and filling of tuber resulted in a large number of tuber with large sizes (Sutater et al., 1993). Apart from how much photosynthates flow, the weight of the potatoes is also related to the number of potatoes that are formed. The more tuber of the same size, the higher the weight of the tuber produced. On the other hand, if a few potato seeds are formed, the photosynthetic flow received by each tile becomes more, causing optimal enlargement of the tubers.

**Percentage of Class L Potato Seeds  $G_0$ .**  
Classification of  $G_0$  potato seeds is based on seed

weight. Class L weight  $> 20 \text{ g}$ , class M seeds weight  $5\text{-}20 \text{ g}$  and class S weight  $< 5 \text{ g}$  (Directorate of Horticulture Seeds, 2014). Table 4 shows that there is an interaction between the K dose and the retardant treatment. Class L seeds with a dose of 150% K ( $d_3$ ) at 100 ppm prohexadione-Ca ( $r_3$ ) were higher when compared to 50% dose K and 100% dose K, but significantly different when compared to other retardant treatments.

This shows that the interaction between the treatment of K fertilizer doses and the concentration of the retardant was able to increase the size of the potato to larger, in this treatment class L potato reached 63.3%. Treatment of 150% dose K plus 100 ppm prohexadione-Ca gave a higher 63.3% percentage of L seed class tuber when compared to other treatments. Element K helps to facilitate the translocation of photosynthetic into the tuber, the more photosynthetic that is produced and absorbed, the larger the potato tuber size could be. Gutomo et al. (2015) suggested that the process of filling potato tubers is related to the photosynthetic ability of plants.

The available K nutrients could increase the rate of photosynthesis as a result of the additional K dose. The photosynthetic results could be stored in food reserves, the photosynthate translocation could be faster when stimulated by giving prohexadione-Ca. This is due to the function of prohexadione-Ca which can increase the rate of photosynthate storage in tuber. This situation can support the growth of tuber so that there are more large tuber due to the more photosynthetic content in these tuber. Most of the results of photosynthesis from plants could flow to plant roots for tuberization such as in potato plants.

**Table 4. Interaction effect of different K fertilizer doses and retardant concentrations on the yield of  $G_0$  potato in the L class seeds**

Dose of K (D)	The L Class Seeds (%)			
	Retardant (R)			
	$r_1$	$r_2$	$r_3$	$r_4$
$d_1$	25.2 a A	44 a B	37.0 a AB	35.5 b AB
$d_2$	23.1 a A	31.7 a A	23.5 a A	23.3 a A
$d_3$	23.3 a A	32.6 a A	63.3 b B	17.8 a A

Note :  $d_1$  =50% dose of K (50 kg KCl/ha),  $d_2$ =100% dose of K (100 kg KCl/ha),  $d_3$ =150% dose of K (150 kg KCl/ha);  $r_1$  = without retardant,  $r_2$  =100 ppm paclobutrazol,  $r_3$  =100 ppm prohexadione-Ca,  $r_4$ = 150 ppm prohexadione-Ca. Means followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally

**Table 5. Effect of different K fertilizer doses and retardant concentrations on time of shoots appear, shoot length, percentage of seeds shoot of G<sub>0</sub> potato**

Treatment	Time Shoots appear (Day)	Shoot Length (mm)	Seed Sprout (%)
d <sub>1</sub> : 50% dose of K (50 kg KCl/ha)	60 a	3.3 a	50.50 a
d <sub>2</sub> : 100% dose of K (100 kg KCl/ha)	60 a	3.6 a	52.00 a
d <sub>3</sub> : 150% dose of K (150 kg KCl/ha)	61 a	2.6 a	53.00 a
r <sub>1</sub> : without retardant	60 b	3.1 a	51.22 a
r <sub>2</sub> : 100 ppm paclobutrazol	61 b	3.5 ab	41.66 a
r <sub>3</sub> : 100 ppm prohexadione-Ca	63 b	2.2 a	56.66 a
r <sub>4</sub> : 150 ppm prohexadione-Ca	54 a	4.0 b	57.77 a

Note : Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level.

**Time Shoots Appear, Shoot Length and Seeds Sprout.** Based on the analysis of variance, it was shown that there was no interaction effect between the dose of K fertilizer and the retardant concentration applied to G<sub>0</sub> seeds at the time of shoot emergence, shoot length and percentage of seeds sprouting. However, independently the retardant concentration treatment showed its effect on the time of shoot emergence and shoot length on G<sub>0</sub> seeds during seed storage. The results of Duncan's multiple range test for the time of shoot emergence due to the application of K fertilizer doses and retardant concentrations are presented in Table 5.

The dose of K fertilizer did not have a significant effect on the time of shoot emergence, shoot length and the percentage of seeds sprouting as can be seen in Table 5. The results of data analysis showed that increasing the number of doses of K fertilizer had no effect on the time of shoot emergence, shoot length, and the percentage of seeds that sprouted during storage.

Giving retardant concentrations to G<sub>0</sub> seeds from cuttings showed a significantly different effect on the time of emergence of shoots. The retardant concentration of 100 ppm prohexadione-Ca (r<sub>3</sub>) required a longer time to produce shoots, namely 63 days after storage, while the concentration of retardant in the treatment of 150 ppm prohexadione-Ca (r<sub>4</sub>) broke dormancy faster, namely 54 days when compared to treatment without retardant, 100 ppm paclobutrazol and 100 ppm prohexadione-Ca. Potatoes could start to sprout after the dormancy period ends. Giving prohexadione-Ca affects the physiological properties of plants which can accelerate the breaking of potato seed dormancy as seen by the emergence of shoots. In line with research conducted by Darmawan et al. (2014) the treatment of prohexadione-Ca can accelerate

flowering by 21 days compared to control and paclobutrazol.

In addition to being influenced by internal factors, the chemical processes that occur during storage are also influenced by environmental factors, namely the temperature of the storage room. Nuraini et al. (2019) stated that one of the factors that caused the length of dormancy was the temperature conditions of storage of potato tubers. Storage of seeds in the medium plains can affect the time of emergence of shoots due to higher temperatures compared to storage in the highlands. High temperatures during the storage of tuber could affect the dormancy of tuber, shorten the resting period, and even accelerate the emergence of shoots (Levy & Veilleux, 2007). Potato seeds could experience a longer dormancy period if stored at 4°C compared to potato seeds stored at 25°C. The average temperature of the storage room in this study was 25°C. The average temperature of the storage room is in accordance with the temperature required for the germination of potato seeds during storage.

Giving the retardant concentration has a significant effect on the length of shoots. The 150 ppm prohexadione-Ca treatment had the highest effect on shoot length and was not significantly different from 100 ppm paclobutrazol while the 100 ppm prohexadione-Ca treatment had the lowest effect. This proves that the treatment of 150 ppm prohexadione-Ca resulted in good potato seed production, which was characterized by higher shoot length growth. The length of the potato shoots is related to the seed dormancy period. Shoots could appear when the seeds have broken their dormancy period. The faster the breakdown of dormancy, the longer the shoots could be, according to the experiment that the 150 ppm prohexadione-Ca treatment gave rise to shoots the fastest, namely 54 days.

Apart from being related to the long dormancy period of the shoots, it is also related to the flow of photosynthetic which is translocated to the tuber. Treatment of 150 ppm prohexadione-Ca can increase photosynthate storage in tuber so they have good vigor. It is this diversion of the flow of photosynthates that can increase food reserves in tuber (Warnita et al., 2019). The higher the content of food reserves contained in tuber, the more shoots that grow could be bigger.

**Tuber Weight Loss.** The results of the statistical analysis showed that there was an interaction between the doses of K fertilizer and the retardant concentration on the percentage of tuber weight loss. The results of Duncan's Multiple Range Test are presented in Table 6.

Based on Table 6, the treatment of 50% dose K ( $d_1$ ) in the treatment of 100 ppm prohexadione-Ca ( $r_3$ ) resulted in 0.42% higher potato weight loss compared to the treatment without retardants, 100 ppm paclobutrazol and 150 ppm prohexadione-Ca and different when compared to the treatment of 100% dose K and 150% dose K. Loss of weight of tuber during storage determines the quality and duration of seeds when stored (Pande et al., 2007). The high temperature and low humidity in the storage room are one of the factors that can increase the transpiration process. Transpiration is the evaporation of water inside the seed to the surface of the seed and from the surface of the seed to the surrounding environment. The loss of water from inside the seed to the surface of the seed results in loss of water from inside the seed which has an impact on decreasing the fresh weight of the seed (Sukarman & Seswita, 2012). Low seed weight loss causes low transpiration and respiration.

Treatment of a 150% dose of K ( $d_3$ ) in the

treatment of 100 ppm prohexadione-Ca ( $r_3$ ) resulted in lower tuber weight loss of 0.11%. This is because the treatment of a 150% dose of K plus a retardant concentration of 150 ppm prohexadione-Ca can suppress the transpiration and respiration processes in  $G_0$  potato seeds from cuttings during storage. Based on research conducted by Oliveira et al. (2021) that prohexadione-Ca can increase the starch and protein content of potato tubers compared to controls. Decreased starch content, increased reducing sugar content, and increased water content in the potato during storage is a form of damage that greatly affects the seeds.

During storage, potato tubers could undergo a metabolic process, namely a process of breaking down starch into sugars, and this process is influenced by the level of respiration rate. The higher the rate of respiration, the faster conversion of starch into sugars. Sugars could be used as energy in the process of respiration (Tronggono, 1990). The water content in potato tubers is also a catalyst in metabolic reactions, therefore fresh potatoes could easily experience quality changes (Winarno, 1980). This is due to the reduced sugar levels that accumulated during storage at cold temperatures being broken down into starch and there is an increase in the respiration and transpiration processes so that the tuber releases water and carbon dioxide into the indoor air. This also causes the weight of the potato tuber to decrease. The low weight loss of tuber in the 150 ppm prohexadione-Ca treatment coupled with a high K dose indicates that the role of K together with prohexadione-Ca is synergistic in facilitating the process of photosynthesis, improving yield quality, reducing the speed of yield decay during transportation and storage (Darmawan et al., 2014).

**Table 6. Effect of interaction between K fertilizer dose and retardant concentration on  $G_0$  seeds from cuttings on tuber weight loss**

Dose of K (d)	Tuber Weight Loss (%)			
	Retardant (r)			
	$r_1$	$r_2$	$r_3$	$r_4$
$d_1$	0.14 a A	0.09 a A	0.42 b B	0.22 a AB
$d_2$	0.36 b B	0.17 a A	0.38 b B	0.13 a A
$d_3$	0.17 a A	0.12 a A	0.11 a A	0.12 a A

Note :  $d_1$  =50% dose of K (50 kg KCl/ha),  $d_2$ =100% dose of K (100 kg KCl/ha),  $d_3$ =150% dose of K (150 kg KCl/ha);  $r_1$  = without retardant,  $r_2$  =100 ppm paclobutrazol,  $r_3$  =100 ppm prohexadione-Ca,  $r_4$ = 150 ppm prohexadione-Ca. Means followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally

## Conclusion

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

1. There is an interaction between K fertilizer dose and retardant concentration on the L seed class and potato weight loss. Treatment with 150% dose K and 100 ppm prohexadione-Ca concentration showed the highest percentage of L seed class and lowest  $G_0$  seed weight loss.
2. The application of K fertilizer independently affects the growth rate of tuber, the number of tuber and the weight of  $G_0$  seed potato. 150% dose of K showed the highest growth rate of tubers and gave the highest number of tubers (6.67 knol/plant) and the highest tuber weight (73.33 g/plant). A concentration of 150 ppm prohexadione-Ca, increased stomatal conductance, and accelerated shoot emergence time and shoot length. The concentration of 100 ppm prohexadione-Ca produced the highest amount of tuber (6.78 knol/plant) and the highest weight of tuber (74.33 g/plant).

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Sari WK · Utami NP

## Successful shoot tip grafting of cacao (*Theobroma cacao* L.) due to the application of plant growth regulators on various concentrations

**Abstract.** Cacao plays an important role in the Indonesia economy. Therefore, its production and area expansion have to increase to achieve maximum cacao productivity by the quality and superior cacao clones. Shoot tip grafting is one of the propagation methods to obtain superior cacao plants by applying Plant Growth Regulators (PGRs). Research on the effect of several PGRs with various concentrations on cacao (*Theobroma cacao* L.) shoot tip grafting was conducted at the Integrated Innovation Farmer Group, Belubus, Sungai Talang Village, Guguak District, Lima Puluh Kota Regency, West Sumatra from February until June 2022. This study aimed to determine the interaction between PGR type and concentration on the grafting success and growth of cacao shoot tip grafted seedling. The research was designed by a Factorial Completely Randomized Design with three replications. The first factor was the type of PGR, i.e., synthetic PGR (BAP), young corn seed extract, shallot extract, and young coconut water. The second factor was the concentration of PGR, i.e., 0%, 25%, 50%, 75% and 100%. The results showed an interaction between PGRs with some concentrations on the number of shoots, shoot length, and leaf length on the grafted cacao seedling. The natural PGR from young coconut water at a concentration of 75% gave the best effect on the number of shoots (3.33 shoots) and shoot length (24.50 cm) of cacao seedling.

**Keywords:** Cacao · Cytokinin · Plant growth regulators · Shoot tip grafting · Young coconut water

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## Introduction

Cacao is an important plantation commodity for the national economy, due to it is the main income for most farmers in Indonesia and potentially developed into the agro-industrial sector. One of the provinces in Indonesia as a center for cocoa production is West Sumatra, however, the cocoa production in this area is still low (productivity of cocoa only reached 200 kg/ha/year). Meanwhile, the average of national cocoa productivity reaches 802 kg/ha/year (Sumilia et al., 2019). It shows that cocoa productivity in West Sumatra has not yet reached the standard of average national cocoa productivity.

The efforts to increase cacao production to achieve maximum productivity should concern to the cacao clones used. The shoot tip grafting method is one way to get superior and quality cacao plants, because there is a combination of different rootstocks and scions with their respective advantages. According to Rahardjo (2011), the advantages of this grafting technique included faster yields and production of plants that are genetically similar with the parent so that cacao plants with uniform productivity and quality will be obtained.

The success of the cacao shoot tip grafting technique is due to the cell response of the scion and rootstock which are interlocked. The treatment that is usually used for the scion of shoot tip grafting is the budding immersion in solutions such as growth regulators. The application of PGRs depends on several factors, such as the duration of soaking in solution and the accuracy of the amount given. Using fewer amounts will cause the effect of PGR to disappear, whereas in high amounts it will inhibit the growth and development of a plant (Gusmawan & Wardiyati, 2019).

Several types of natural PGRs contain the hormones of cytokinin, auxin, and gibberellin, which can support the success and growth of shoot tip grafting of cacao. Types of plants that contain bioactive compounds that can be used as extraction materials as growth regulators include compound extracts from young corn seeds, shallots and young coconut water. Pagalla et al. (2015) detected that corn seed extract contained PGRs, namely zeatin (cytokinin) 53.94 ppm, auxin 1.67 ppm and gibberellin 41.23 ppm. In addition, Trifaldi (2021) found that young

corn extract at a concentration of 75% has the best effect on shoot length and leaves number of side grafting of cacao.

According to Kurniati et al. (2019), shallots is a natural PGR that contains the hormone auxin (156.01 ppm in the form of IAA), cytokinin (zeatin: 122.34 ppm and kinetin: 140.11 ppm) and gibberellin (230.67 ppm). The research of Darajat et al. (2014) revealed that soaking cacao seeds in shallot extract at a concentration of 10% was able to significantly increase the percentage of germination, growth speed, and the length of hypocotyl and root.

Furthermore, Djamhuri (2011) revealed that young coconut water contains the hormone gibberellins (0.460 ppm GA<sub>3</sub>; 0.255 ppm GA<sub>5</sub>; 0.053 ppm GA<sub>7</sub>), cytokinin (0.441 ppm kinetin; 0.247 ppm zeatin) and auxin (0.237 ppm IAA). Application of young coconut water at a concentration of 75% has an effect on the number of leaves and stem diameter of cocoa seedlings (Yassi et al., 2016). Moreover, Putri et al. (2021) found that application of coconut water at a concentration of 75 - 100% increase the growth of cacao seedlings especially on plant height, stem diameter, leaf area, and dry weight.

It is presumption that there is an interaction effect between the natural PGRs types and their concentrations, therefore the objective of this study was to determine the interaction effect between those two factors on the success and growth of shoot-tip grafted cacao seedling. The finding of present study will provide valuable information regarding the potential use of natural PGRs which are more affordable and environmental-friendly than the synthetic ones for boosting cacao seedling production.

## Materials and Methods

This research was carried out on February until June 2022 at the Integrated Innovation Farmer Group, Belubus, Sungai Talang Village, Guguk District, Lima Puluh Kota Regency, West Sumatra. The materials used in this study were MCC 02 cacao seedlings as rootstock, BL 50 cacao clone as scion, extract natural PGRs (young corn seed extract, shallot extract, and young coconut water), synthetic PGR (Benzyl Amino Purine/BAP), distilled water, label paper and plastic bags. The tools used in this study were grafting knives, blenders, filters for natural

PGR, containers, measuring cups, shading net 75%, tape measure, rulers, camera, and stationery.

This study was arranged in a Factorial CRD consisting of two factors which were repeated three times. The first factor is the type of PGRs, namely:

- T0 : synthetic PGR (BAP)
- T1 : young corn seed extract
- T2 : shallot extract
- T3 : young coconut water

The second factor is the concentration of each PGR given, consisted of 5 levels, namely:

- C0: 0% (without PGR)
- C1: 25% (25 ml PGR + 75 ml water)
- C2: 50% (50 ml PGR + 50 ml water)
- C3: 75% (75 ml PGR + 25 ml water)
- C4: 100% (100 ml PGR)

Observed data were analyzed by analysis of variance at the 5% level of significance. If the results are significantly different ( $F_{count} > F_{table}$ ), continue with Duncan's New Multiple Range Test (DNMRT) at the 5% level.

The research was begun by the preparation of location and MCC 02 cacao seedlings, which were 4 months old as rootstock (seedlings healthy, free from pests and diseases, fresh green leaf color, minimum number of 6 leaves, and stem diameter of  $\pm 1$  cm). Then, in preparation for the scion in the afternoon, it was a BL 50 clone derived from a tree that was 10 years old. The production branch used as the 2.5-month-old scion was taken in the middle part of the branch where all the leaf stalks were cut, the color was brownish green and the scion length was 10 cm with 3 buds.

Natural PGR is prepared from young corn seed extract, shallot extract, and young coconut water. It was pureed with a blender without adding water then filtered to separate the liquid extract from the pulp. The concentration of each PRG stock solution was 100%, and then it was made according to each treatment. PGR application was carried out before grafting by immersion of the cacao shoots/scion for 12 hours in a PGR solution according to each treatment.

The V-grafting technique carried out the shoot tip grafting. The rootstock seedlings (4 months old) were cut at a height of 25 cm, and the rootstock was split from top to bottom along 3 cm. Meanwhile, the scion used had been treated with PGR, and then the scion was sliced to form V with an even incision area.

Furthermore, the grafts are inserted into the rootstock gap that has been prepared and tied with grafting plastic. Watering once a day. In addition, the shoots that grown on the rootstock are removed to avoid competition for scion growth. Finally, the bond graft was opened about three months after grafting.

Observed variables were the age of shoot emergence, number of shoots, shoot length, number of leaves, leaf length and width were measured on a same leaf which is the longest leaf at the beginning of the observation, percentage of success graft and percentage of survival graft.

## Results and Discussion

**The Age of Shoot Emergence.** The results of analysis of variance showed that there was no interaction effect between the application of natural PGR at various concentrations on the age cacao shoots appeared. However, it was affected only by the application of PGR and was not affected by its concentration given (Table 1).

**Table 1. The independent effect of several PGRs with various concentrations on the age of shoot emergence (days) of cacao shoot tip grafting**

PGR Types	Concentration of PGR (%)					Mean
	0	25	50	75	100	
T0	17.16	19.16	18.33	22.50	17.00	18.83 a
T1	18.33	17.00	16.50	18.16	21.33	18.27 a
T2	13.00	14.16	11.16	15.83	16.50	14.13 b
T3	18.22	15.83	19.00	18.50	18.50	18.03 a

Coefficient of Variance = 8.44 %

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

Table 1 showed that the natural PGRs from shallot extract (T2) generates the fastest cacao shoots emergence (14,13 days after grafting). The application of synthetic PGR (T0) and natural PGR from extracts of young corn seeds (T1) and young coconut water (T3) had the same effect on the age of shoot emergence of cacao shoot tip grafting.

According to Nur et al. (2023), the average emergence of buds on shoot tip grafting of cacao plants is around 30 days. This indicates that the growth regulators in the present study able to accelerate the emergence of shoots, and natural growth regulator from shallot extract was better

than others, because the content of cytokinin hormone in shallot extract is more than on young corn seed extract and young coconut water. It is supported by Kurniati et al. (2019) revealed that shallot is a natural growth regulator that contains a cytokinin of 262.45 ppm. Meanwhile, the cytokinin content in young corn seed extract is 53.94 ppm (Pagalla et al., 2015) and 0.688 ppm in young coconut water (Djamhuri, 2011).

Growth regulators are organic compounds that affect physiological processes, such as growth, cell differentiation and plant development (Asra et al., 2020). Applying natural PGR on cacao shoot tip grafting gave a better effect on the age of shoot emergence compared to synthetic growth regulators (BAP). Besides that, utilization of natural growth regulators can support earth conservation because it being environmentally friendly. It was propped by Pinto et al. (2012) that the application of natural growth regulators does not leave residues that disturb the environment and human health.

**Number of Shoots.** Based on the observation of the number of shoots on 12 weeks after shoot tip grafting, it indicated that there was an interaction between the PGR types and their concentrations (Table 2). It shows that the average number of shoots on 12 weeks after grafting ranges from 2.00 – 3.33 shoots. Application of natural growth regulators from young coconut water (T3) with a concentration of 75% showed the best results for the number of grafted shoots (3.33 shoots).

**Table 2. The interaction effect of several PGRs with various concentrations on the number of shoots at 12 weeks after shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	2.00 a	2.83 a	2.16 a	2.33 b	2.00 b
	B	A	AB	AB	B
T1	2.16 a	2.00 b	2.00 a	2.83 ab	2.33 ab
	AB	B	B	A	AB
T2	2.00 a	2.33 ab	2.33 a	2.50 b	3.00 a
	B	AB	AB	AB	A
T3	2.00 a	2.00 c	2.50 a	3.33 a	2.83 a
	C	C	BC	A	AB

Coefficient of Variance = 7.82 %

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

Application of young coconut water PGR at a concentration of 75% was significantly different with other concentrations (0%, 25% and 50%). However, it had the same effect with the concentration of 100% on the number of shoots. At a concentration of 75%, utilization of young coconut water PGR was also significantly different from the synthetic PGR (T0) and PGR from shallot extract (T2), but had the same effect with young corn seed extract (T1).

Application of young coconut water as growth regulators at a concentration of 75% can increase the number of shoots because of the presence of growth hormones i.e., auxins and cytokinins that stimulate shoot growth if given in the precise amount and concentration. It contains cytokinin hormone 5.8 mg/L that stimulate shoot growth and encourage the activities of living cells or tissues, the auxin hormone 0.07 mg/L and a few gibberellins as well as other compounds that can stimulate germination and growth (Setiawan et al., 2013).

Asra et al. (2020) revealed that the increase of the number of shoots is related to the function of cytokinins in the process of cell division, formation of plant bud and organ. In addition, auxin plays a role in mobilizing cells to form new shoots, because auxin can induce rapid cell enlargement due to the activation of ATP-ase which pumps protons in the cell membrane that cause activation of cell expansion (increase the number and size of cells).

A concentration of 75% is the right amount that needed by cacao shoot grafting in the formation of shoots. This is in accordance with Pamungkas et al. (2020) stated that growth regulators are only effective in certain amounts, while the mistake amount will result in stunted plant growth.

**Shoot Length.** Analysis of variance results on the shoot length on 12 weeks after grafting showed that there was an interaction between the application of some types growth regulators with various concentrations on cacao shoot tip grafting (Table 3). The high shoot growth that occurs is due to the right interaction between those.

Table 3 shows that the average shoot length ranges from 12.16 – 24.50 cm and 75% young coconut water PGR gave the best results for shoot length (24.50 cm). Utilization of growth regulators (PGR) of young coconut water at a concentration of 75% had a highly significant different effect with synthetic ZPT/BAP (T0),

young corn seed extract (T1) and shallot extract (T2). Likewise, its application also had a significant different effect with others concentrations (0%, 25%, 50% and 100%) on the shoot length.

**Table 3. The interaction effect of several PGRs with various concentrations on the shoot length (cm) at 12 weeks after shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	14.83 a AB	19.00 a A	15.00 ab AB	17.00 b AB	13.16 ab B
T1	17.08 a A	17.83 a A	18.33 a A	16.00 b AB	12.16 b B
T2	13.00 a B	14.50 a AB	12.66 b B	18.33 b A	13.66 ab B
T3	14.16 a B	16.16 a B	16.50 ab B	24.50 a A	17.41 a B
Coefficient of Variance = 6.97 %					

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

This is in line with Yassi et al. (2016) that giving young coconut water at a concentration of 75% has the best effect on the growth of cacao seedlings. According to Manurung et al. (2017), young coconut water can encourage shoot growth because it contains the cytokinin hormone of 5.8 mg/L higher than the auxin content which only 0.07 mg/L. It absorbed by plant tissues to activate food reserve energy which can encourage cell division and tissue differentiation, which forms buds and plays on shoot elongation.

On the other hand, at higher concentrations auxin can inhibit cell elongation. The shoot growth occurs is due to the proper interaction between the added exogenous hormones. The balance of auxin and cytokinin concentrations added resulted in good shoot growth (Manurung et al., 2017). The auxin content on young corn and shallot extract are relatively high that causing the shoots growth slower than shoots given young coconut water.

**Number of Leaves.** Table 4 shows that the average number of leaves on shoot grafting ranged 6.50 – 16.66 leaves and the application some growth regulators with various concentrations did not affect the number of leaves on cacao shoot tip grafting, automatically there is no interaction between them.

The number of leaves were not significant in this study suspected because the erratic

weather when the plants generate leaves. Most of the leaves fall at transition from rainy season to the dry season. The monthly rainfall when the research was carried out (February – June 2022) was ranges from 205-260 mm which is the medium category (Center for Water Resources Management Limapuluh Kota Regency, 2022). Besides that, intercepted sunlight is around 25% can stimulate the activity of hormones that encourages the cell division which has a direct effect on leaf growth. On the other hand, Rosniawaty et al. (2020) revealed that coconut water at a concentration of 50% was able to increase the growth of cacao seedlings of the ICCRI 08 H cultivar and showed the best effect on the variable number of leaves.

**Table 4. The independent effect of several PGRs with various concentrations on the number of leaves on 12 weeks after shoot tip grafting.**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	11.33	9.50	10.16	6.50	10.16
T1	10.50	13.16	13.16	9.33	7.83
T2	13.50	16.66	7.66	7.33	13.16
T3	12.83	13.00	11.00	10.50	12.00
Coefficient of Variance = 17.49 %					

Cacao is classified as C3 plants which able to photosynthesize at low temperatures. Excessive photosynthesis will be cause leaf fall, whereas high temperatures over a long period will cause the shoots death. In addition, the increase in leaves is influenced by genetic and environment where the leaf blade will develop according to a certain pattern according to its habitus and will stop at the maximum leaf size limit (Gusmawan & Wardiyati, 2019). This is in accordance with the opinion of Wulandari et al. (2013) that the number of leaves is influenced by genotype and environment, on relatively constant environmental conditions enabling leaf primordial to grow with a constant growth rate.

**Leaf Length.** The linkage between the scion and rootstock will affect the growth of the grafted leaves, where the leaves as a producer of energy and food reserves (photosynthates) for plant growth and development. Based on the analysis of variance, it indicated that there was an interaction between the types of growth regulators with various concentrations on the leaf length of cacao shoot tip grafting as present in Table below.

Table 5 shows that the effect of some PGR on the average of cacao leaf length aged 12

weeks after grafting ranged from 11.08 – 21.33 cm. Application of young corn seed extract (T1) at a concentration of 75% was shown the longest leaf length (21.33 cm), although the effect was the same with synthetic PGR (T0) and shallot extract (T2). Otherwise, it was significantly different effect with concentrations of 0% and 100%, but had the same effect with concentrations of 25% and 50% on the leaf length of grafted cacao shoots.

**Table 5. The interaction effect of several PGRs with various concentrations on the leaf length (cm) at 12 weeks after shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	16.25 ab AB	18.50 a AB	15.50 b B	18.00 ab AB	20.50 a A
T1	15.33 bc BC	17.58 ab AB	17.75 a AB	21.33 a A	11.08 c C
T2	17.75 a A	17.41 ab A	15.66 b A	16.08 ab A	16.91 b A
T3	14.75 c A	15.16 b A	16.83 ab A	14.58 b A	16.83 b A

Coefficient of Variance = 10.45 %

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

The results of this study are in line with Trifaldi (2021) that the highest value of leaf length (26,94 cm) on the side-grafted cacao was obtained from the application PGR at a concentration of 75%. This is because the leaf growth is strongly influenced by the genetic nature of the plant. The scions for grafting in the present study was BL 50 clone, where it has the characteristic shape of leaves that are long and pointed.

**Leaf Width.** Application of some growth regulators at various concentrations did not affect the leaf width on cacao shoot tip grafting at 12 weeks after grafting (Table 6). The average leaf width ranges 4.75 – 7.25 cm that were relatively the same each other's. This is in accordance with the opinion of Trifaldi (2021) that the average leaf width is relatively the same due to the same genetic characteristics, as the scions used in this research was only one type, namely BL 50 clone.

The effect of growth regulators, such as cytokinin has not been able to support the growth of leaf width, suspected that it used for the growth of other grafted parts, such as shoot length and number of leaves. Provision of

growth regulators will be effective if used at certain growth phases and under the supportive environmental conditions. Moreover, according to Sumilia et al. (2019), the light requirement that can be sufficient for the assimilation process of the cacao plant is around 75% of the total full sunlight. Sunlight will affect photosynthetic activity and the speed of plant growth in leaves. The higher the photosynthetic activity, the growth of leaves will also increase and bigger. Sunlight also stimulates the activity of endogenous hormones that encourages the process of cell division, enlargement and forms new cells that affect the leaf width.

**Table 6. The average cacao leaf width (cm) on 12 weeks after shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	5.75	7.25	6.33	6.45	7.16
T1	6.58	6.41	6.91	6.75	4.75
T2	7.08	7.00	5.66	6.91	6.66
T3	6.00	6.25	5.83	6.50	6.91

CV = 17.10 %

**Percentage of Success Graft.** Based on analysis of variance noted that application of some PGR with various concentrations had no effect on the percentage of success cacao shoot tip grafting. Observation data on the average percentage of success graft show in Table 7.

**Table 7. The average percentage of success (%) of cacao shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	100	66.67	100	83.33	83.33
T1	83.33	100	83.33	66.67	83.33
T2	100	100	83.33	83.33	83.33
T3	100	100	66.67	100	83.33

CV = 15.56 %

Table 7 shows that the success rate of cacao shoot tip grafting ranged from 66.67 – 100%. It was quite high if compared to previous research conducted by Limbongan & Djufry (2013) who reported that the success percentage of cacao side grafting was around 69.90 – 75.40%, and even decreased by 2.00 – 41.80% due to high rainfall after grafting. Many things can affect the success rate of grafting, including the skill of the person doing the grafting, the time of grafting, the weather conditions, and the compatibility between the rootstock and scion.

This was confirmed by Trifaldi (2021) who stated that the compatibility between scion and

rootstock involves a relationship between them, even though the varieties are different, but if they are still in the same genus, so compatible. Nurhalim & Nuha (2022) also revealed that scion and rootstock relationships affect nutrient distribution patterns, the ability of nutrients to move across the graft link and regulation of hormone transport. The use of growth regulators which contain the hormone cytokinin (zeatin) also provides the same nutrition for the linkage process of the scion with the rootstock.

**Percentage of Survival Graft.** Table 8 shows that the application of growth regulators with various concentrations did not significantly affect the percentage of survival cacao grafting. The average percentage of survival grafting ranged from 33.33 – 83.33 %. The low percentage of survival grafting is thought due to grafting was carried out in the rainy season, then during shoot growth there was a transition from the rainy season to the dry season (monthly rainfall ranges from 205-260 mm), causing abnormal grafting growth and lots of fallen leaves.

**Table 8. The average percentage of survival (%) of cacao shoot tip grafting**

PGR Types	Concentration of PGR (%)				
	0	25	50	75	100
T0	66.67	66.67	83.33	66.67	50.00
T1	83.33	83.33	83.33	66.67	33.33
T2	50.00	66.67	50.00	83.33	50.00
T3	50.00	66.67	50.00	83.33	66.67
CV = 25.64 %					

According to Trifaldi (2021) the high risk of death for scion stems is due to the penetration of rainwater at the connection (enclosed part) causes decay (death) of cells or plant tissue there. In addition, covering the buds can also increase moisture so that the risk of fungal attack on the joints also increases. Fungus attack on shoots is characterized by symptoms of wilting and drying.

The average percentage of survival was highest at a concentration of 75%, while the lowest was at a concentration of 0% and 100%. This is presumably because growth regulators at certain concentrations can increase the percentage of survival grafting. As stated by Nurlaini & Imam (2015) that utilization of PGR at the right concentration can increase the growth percentage, while at the wrong concentration cause stunted or abnormal plant growth.

## Conclusion

1. There was an interaction effect between the types of PGR with their concentrations on variables of the number of shoots, shoot length and leaf length of cacao shoot tip grafting.
2. Natural growth regulators from young coconut water at a concentration of 75% had the best effect on the number of shoots and shoot length of cacao shoot tip grafting.

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Ruminta · Maysix L · Wicaksono FY

## Identification of climate change and its impact on maize (*Zea mays* L.) production in Majalengka Regency

**Abstract.** Maize is one of the multipurpose crop whose yields can be affected by climate change. Climate change includes increasing air temperature, increasing sea levels, and changes in rainfall can be a threat and cause a decrease in maize yields. This could be caused by climate change factors. This research aims to identify climate change and analyze the correlation between climate change and maize production in the Majalengka Regency. Maize plantations in the 26 sub-districts are still fluctuative. The method used in this research is quantitative descriptive using trend analysis and correlation. The data used include temperature, rainfall, harvested area, production, and productivity of maize obtained from (i) the Meteorological, Climatological, and Geophysical Agency of Majalengka Regency, (ii) Statistics Indonesia, (iii) Food Security, Agriculture, and Fisheries Office of Majalengka Regency, and other related sources. The results of this research showed that climate change in Majalengka Regency was marked by an increase in temperature trend and a decrease in rainfall trend. The correlation between temperature and changes in maize production in Majalengka Regency was significant, while rainfall was not significant.

**Keywords:** Global warming · Rainfall · Temperature

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## Introduction

Maize (*Zea mays* L.) is the most important cereal crop because its utilization is relatively diverse and its use supports agriculture zero waste (Panikkai et al., 2017). Maize is used as a source of food, such as the people of East Nusa Tenggara who make maize as their staple food (Yusuf et al., 2013). Besides that, maize can be used as feed, fuels, and other industrial raw materials.

Maize has a multipurpose function and is followed by an increasing demand every year. However, maize often experiences production fluctuations. This can be seen from the frequent fluctuations in maize production in West Java from 2019 to 2021, with recorded production of 1.3 million, 1.4 million, and 1.1 million tonnes (Department of Food Crops and Horticulture, 2021).

In the cultivation of maize, climate is an influential factor in its growth and development. Temperature can affect the physiological processes of crop (Herlina & Prasetyorini, 2020). The optimum temperature for the growth of maize crop is 23 – 27 °C, while the minimum temperature for maize growth is 8 – 10 °C and the maximum temperature is 40 °C (Amaru et al., 2013). Rainfall supports the availability of water for crop (Aqil et al., 2007). Increased rainfall can cause flooding, while decreased rainfall can cause drought which can reduce agricultural production. Lack of water in maize crops can reduce maize yields by 15% especially drought during the flowering stage and seed-filling stage (Muhadjir, 2018). Besides that, sunlight helps in the process of photosynthesis. Maize crops need direct sunlight in the process of growth and development.

Nowadays, the climate has changed. Climate change is a condition of climate components where there is a change that deviates from the usual average state. The causes of climate change can occur due to human activities (anthropogenic) so the contents of the atmosphere (the gas layer that protects the planet Earth) experience changes and cause climate change. According to Julismin (2013) climate change in Indonesia is increasingly being felt by the increasing frequency of floods and droughts. In addition, according to the IPCC report in Ridha et al. (2016) the earth's temperature has increased by around 0.8 °C for

the last 100 years. The study is supported by studies on climate change in the Malang Region, East Java, with the result that air temperature has increased by 0.7 – 0.8 °C and rainfall decreased by around 0 – 550 mm which resulted in Oldeman's classification changing from class C3 to C2 as well as in the South Sumatra region which showed the results of air temperature increasing by 0.4 – 0.6 °C and rainfall decreased by 0 – 197 mm (Ruminta et al., 2018).

If the weather is unsuitable for maize crop, it can make maize productivity decrease. Climate change can occur anywhere, even in Majalengka Regency. Majalengka Regency is one of the maize centers in West Java. Based on the Agriculture and Fisheries Food Security Service of Majalengka Regency (2021) maize production has fluctuations especially from 2017 until 2021, namely 151,646, 138,074, 146,130, 150,647, and 118,795 tons. The increase and decrease in maize production in Majalengka Regency is suspected to be partly due to climate change. Various regions in Majalengka Regency have different microclimates because their topography varies, from lowlands to highlands. This causes differences in microclimate, such as cooler temperatures in highland areas and hotter in lowland areas. The magnitude of the consequences of climate change is felt necessary conducted a study on the estimated impact of climate change on maize production in Majalengka Regency in 1990 – 2021. The purpose of this research is to identify climate change, especially temperature and rainfall in Majalengka Regency and to find out the correlation between climate change and maize production in Majalengka Regency in 1990 – 2021.

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## Materials and Methods

This research was conducted from October 2022 - January 2023 by quantitative descriptive method. This research collected several data using observation techniques, archival data, and surveys in the form of climate data including temperature and rainfall data as well as maize crop data including planted area, harvested area, production, and productivity each obtained from 1990 – 2021. Climate data were obtained from Meteorological, Climatological, and Geophysical Agency Majalengka Regency, while data for maize farming were obtained from (i)

Statistics Indonesia, and (ii) Food Security, Agriculture, and Fisheries Office of Majalengka Regency. Analysis of research data using trend and correlation analysis is as follows

(a). Trend analysis uses the following equation:

$$Y = b_0 + b_1X$$

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

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

where: Y = trending value of climate data (rainfall or temperature) or maize crop data (production and productivity);  $b_0$  = constant value, that is the value of Y when the value of X = 0;  $b_1$  = The value of the slope of the line, that is the additional value of Y, if X increases by one unit; and X = Year period value

(b). Analysis of the correlation between climate change and changes in maize production uses the following formula:

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

where:  $r$  = correlation coefficient;  $X_i$  = data on rainfall or temperature;  $Y_i$  = maize production or productivity data.

Processing and analysis of research data using software Minitab 19, SPSS 24, and Microsoft Excel 2019

## Results and Discussion

### Climate Description of Majalengka Regency.

The climate in Majalengka Regency is included in the tropical climate, that is, there are two seasons in one year which consist of a rainy season and a dry season. Throughout 1990 - 2021 Majalengka Regency has an average amount of rainfall of around 2656 mm/year which is included in the pattern group monsoonal, that is during the rainy season and dry season there is a

clear difference (Hermawan, 2010) and the humidity is around 79%. In addition, temperatures have varied in Majalengka Regency over the last 32 years around 26.7 - 28 °C with an average temperature of 27.33 °C.

**Analysis of Climate Change in Majalengka Regency.** Based on the results of the analysis of temperature and rainfall data for the last 32 years, between period 1 (1990 - 2005) and period 2 (2006 - 2021) in Majalengka Regency, there have been changes as shown in Table 1. Every period consists of 15 years because seeing climate change takes a long time.

Table 1 shows that Majalengka Regency experienced climate change between periods 1 and 2 as indicated by the air temperature increasing by 0.33 °C. In addition, the average amount of rainfall between periods 1 and 2 decreased by 56.4 mm. The increased temperature can be supported by the results of calculating dry months in both periods where the number of dry months in period 2 is more. Indonesia has experienced an average temperature increase of around 0.1 °C/year since the 1990s (Febrianti, 2018) and according to Runtunuwu & Syahbuddin (2007) global temperature has increased over a period of 95 years by 0.57 °C. In the Oldeman climate classification type, Majalengka Regency has not changed, that is type D3. The number of Wet Mont and Dry Mont respectively in period 1 was four each, while in period 2 there were four and five. Therefore, the D3 Agroculture Zone in the Majalengka Regency area is suitable for planting once rice crop and once palawija crop, such as maize, but still attention guaranteed water availability throughout the year (Anwar et al., 2019).

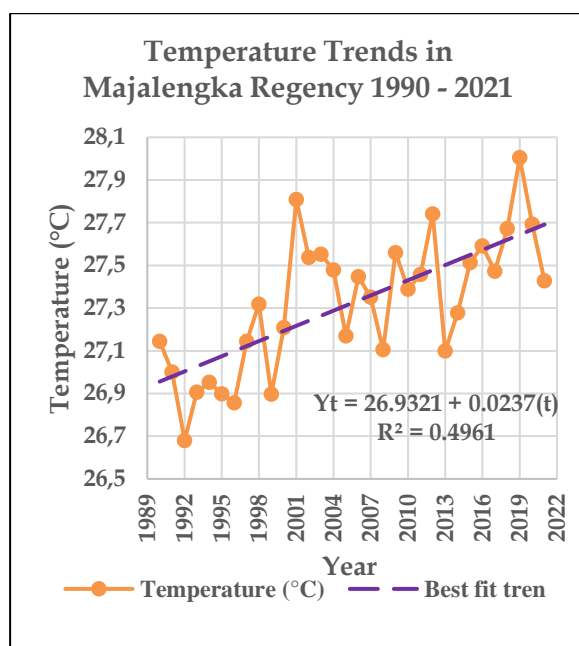
**Table 1. Climate change in Majalengka Regency**

Climate Indicator	Climate change		Magnitude of Change
	Period 1	Period 2	
Average Temperature (°C)	27.16	27.49	0.33 (+)
Average Amount of Rainfall (mm)	2683.8	2627.4	56.4 (-)
Wet Months	4	4	0
Dry Months	4	5	1
Oldeman Climate Classification Types	D3	D3	No changes

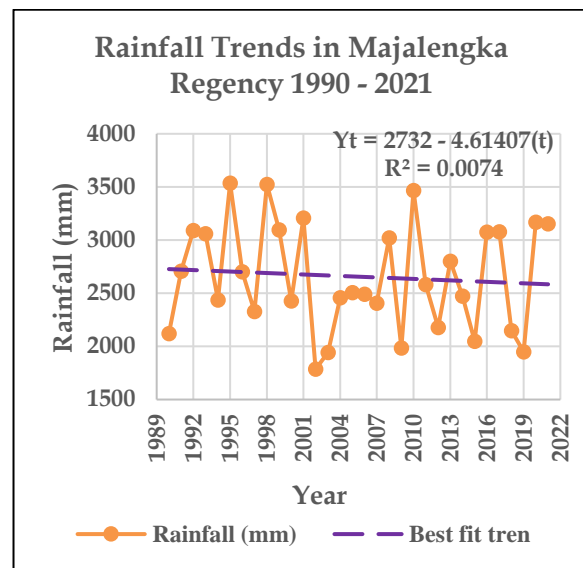
Trend analysis is carried out to determine the pattern of tendencies (trends) of a change (Susilokarti et al., 2015). Based on trend analysis, temperature has increased, while rainfall has

decreased (Figures 1 and 2). The increase in temperature can be affected by the number of population. In 2019 the population in Majalengka Regency reached 1,205,034 people with an average population density of 1,000.66 people/km<sup>2</sup>. The population is in line with the increase in greenhouse gas emissions which can increase air temperatures (Maria, 2021). In addition, it was followed by various developments, such as the construction of industrial land, infrastructure, residential areas, and the existence of the Kertajati International Airport (Pramudiyasari et al., 2021). This can affect global warming. Global warming is an increase in global average temperature caused by the emission of greenhouse gases into the atmosphere which has a lot and traps solar heat energy (Kusumawardhani & Gernowo, 2015).

The graph of the rainfall trend shows that the amount of rainfall in Majalengka Regency is relatively varied and tends to decrease. Changes in rainfall can be affected by Monsoon Australia – Asia and El Nino-Southern Oscillation (ENSO) which has an impact, such as increasing extreme climate events, such as floods and droughts. El Nino is a condition of below normal rainfall, while La Nina is a condition of above normal rainfall (Sitompul & Nurjani, 2013). Along with climate change, the El-Nino period is becoming faster, becoming 2 - 3 years, from 5 - 6 years at first (Ruminta et al., 2018). During an El Nino, maize production can decrease by around 7.4% during an El Nino (Santoso, 2016).



**Figure 1. Graph of air temperature trends in Majalengka Regency 1990 - 2021**



**Figure 2. Graph of rainfall trends in Majalengka Regency 1990 - 2021**

**Analysis of Changes in Maize Production in Majalengka Regency.** From the graphic image of the trend maize production in Majalengka Regency 1990 - 2021, it shows a line that tends to increase (Figure 3). This is because farmers generally have implemented maize cultivation properly, such as using certified seeds and planting 1 - 2 seeds/hole. Planting maize with 1 seed/hole will relatively give good results because it reduces competition, such as space, water, light, and nutrients between crops (Bolly, 2018). The use of hybrid seeds has also been widely applied, such as the Bisi 18 and NK 212 varieties which are drought resistant and capable of producing an average yield of 9.1 and 9.5 t/ha of dry shell (Cereal Crops Research Institute, 2012). Hybrid seeds have better quality than composite seeds which are usually used by farmers before using hybrid seeds. This is because hybrid seeds have dominant genes that give high yields (Haryati & Permadi, 2015). In addition, the temperature in the last 32 years shows values in the range of 26.7 - 28 °C and this shows a sufficient temperature for the growth and development of maize crop which has an optimum temperature of 23 - 27 °C (Amaru et al., 2013). The optimum temperature is the best temperature for ideal crop growth.

On the other hand, maize production has often decreased. In previous observations it was seen that rainfall was decreasing and this had

the potential to cause drought. Drought can affect the physiological processes of maize crop, such as the process of photosynthesis, growth, the seed filling phase is hampered, and the weight of maize kernels decreases and the size of the seeds decreases (Hirich *et al.*, 2012). When the temperature exceeds the suitable for maize crop it will give different results (Herlina & Prasetyorini, 2020).

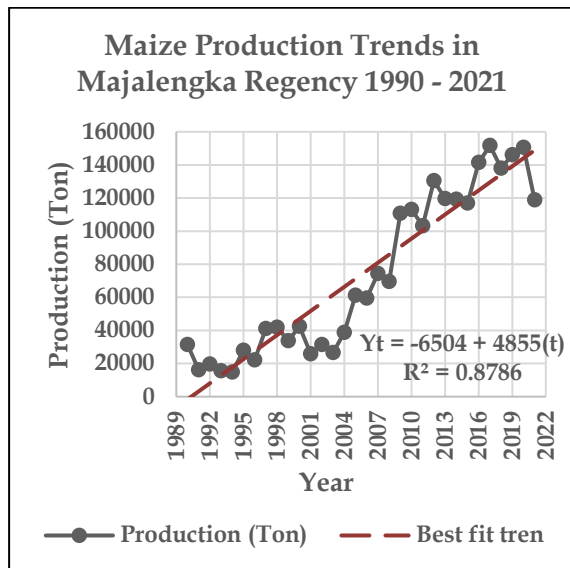


Figure 3. Graph of maize production trends in Majalengka Regency 1990 - 2021

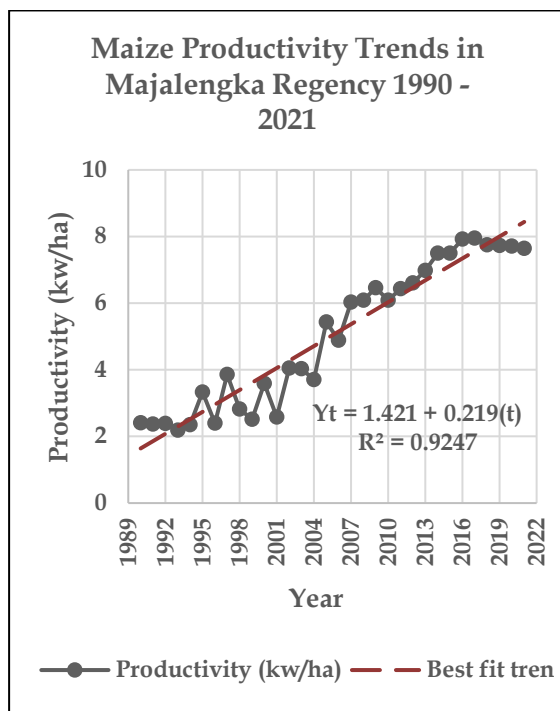


Figure 4. Graph of maize productivity trends in Majalengka Regency 1990 - 2021

Not only on production, analysis of the maize productivity was also carried out (Figure 4). Maize productivity is influenced by production yields and maize crop area. Productive land use can determine the productivity of agricultural commodities, especially food crops, which are a source of food security in West Java. The area planted for maize still fluctuations, for example from 2017 until 2021, namely 21.054, 15.967, 18.120, 19.477, and 15.909 ha. One of the challenges currently faced in agricultural cultivation activities is land conversion. This is also a challenge in the use of agricultural land in Majalengka Regency with higher population growth which can change the local environment. This can be seen in the use of agricultural land in 2020 which has decreased compared to previous years to reach 371 Ha. One of them is the Development of the West Java International Airport (BIJB) in Majalengka Regency which has resulted in the conversion of agricultural land so that farmers lose their livelihoods and change farmers income (Hidayat *et al.*, 2017). With a variety of human activities, it can add greenhouse gases which will accumulate in the atmosphere and trigger global warming (Aldrian *et al.*, 2011).

**Correlation of Climate Change Relationship with Changes in Maize Production in Majalengka Regency.** Maize yields are influenced by two factors, biotic factors and abiotic factors including pests, diseases, weeds, soil microorganisms, soil, and climate. Changing climatic conditions will affect the production of a crop, as well as the maize crop (Nurmala *et al.*, 2015). The analysis technique commonly used to determine the strength and direction of the linear relation of two variables is the Pearson correlation analysis technique. Pearson correlation analysis was used to determine whether there is a relationship between changes in temperature and rainfall on changes in maize production and productivity. The results of the correlation analysis between changes in temperature and rainfall on maize production and productivity in Majalengka Regency are shown in Table 2.

Table 2 showed temperature changes were significantly correlated with a positive direction, while changes in rainfall were not significantly correlated with a negative direction on maize production and productivity. Changes in



temperature with a significant correlation illustrate that temperature changes affect changes in the production and productivity of maize. Based on previous study by Herlina & Prasetyorini (2020), temperature and productivity have a relation. The positive direction indicates that when the temperature increases, maize production and productivity also increase to a certain extent (23 – 27 °C). This is because temperature can activate enzyme performance, when temperature increases, enzyme performance increases, and causes the rate of photosynthesis to increase (Su'udi et al., 2022). The rate of biological metabolism increases as the rate of enzymes increases, such as the Rubisco enzyme in the photosynthesis process. The rubisco enzyme catalyzes the reaction of adding CO<sub>2</sub> to RuBP and produces 3-PGA. This is a step in converting light energy into chemical energy that crops can need. Rubisco enzyme efficiency has a direct impact on photosynthesis rates and crop productivity (Moore et al., 2021). If the temperature is not normal for maize crop, the increase in maize production will be hampered.

**Table 2. Correlation of changes in maize production with climate in Majalengka Regency**

Correlation	Temperature	Rainfall
Production	0.637*	-0.225
Productivity	0.628*	-0.278

Note: (\*) significant

Changes in rainfall with no significant correlation indicate that changes in rainfall don't directly affect changes in the production and productivity of maize. This is because even though rainfall tends to decrease, it is enough to meet the water needs of maize crop. Based on previous study by Herlina & Prasetyorini (2020), rainfall doesn't have a significant correlation with maize productivity. The resulting negative direction illustrates that when rainfall decreases, the production and productivity of maize crops will increase. This is because when the rainy season coming, farmers in Majalengka Regency prefer to plant rice crop. After all, this crop can survive in flooded conditions. High rainfall can cause puddles in maize planting land resulting in abiotic stress. Stagnant water in maize crop can reduce production by 30 - 50% from normal conditions (Li et al., 2011). Conversely, when the dry season enters, farmers prefer to plant maize crop

(Ekopranoto, 2019). Rainfall tends to decrease causing clouds to rarely appear so that sunlight can be maximally received by the earth. The intensity of sunlight that is fully captured by maize crop can increase the rate of photosynthesis of these crops (Yustiningsih, 2019). Sunlight is the main energy source in the photosynthesis process in converting water and CO<sub>2</sub> into oxygen and glucose. Leaves absorb 1 – 5% of solar energy and with sufficient water they can optimize the use of sunlight in the photosynthesis process. If there is water stress, the stomata tend to close in response to reduce water evaporation (Taiz & Zeiger, 2010).

There is also a correlation analysis between temperature and maize production and productivity in 26 sub-districts in Majalengka Regency which is contained in the form of a map. (Figure 5 and Figure 6). There are various colors on the map which reflect that the darker the color, the stronger the relationship between these variables, and the lower the relationship, the more faded the color. A positive correlation or a one-way relationship between variables is depicted in green on the map, while a negative correlation or a relationship in the opposite direction is depicted in blue on the map. The results of the correlation analysis in Figure 5 show a positive correlation that dominates, meaning that an increase in temperature will be in line with an increase in maize production in the sub-district and various relationship categories. The very low category correlation includes the Sub-Districts of Sumberjaya, Palasah, Sukahaji, and Banjaran with a coefficient value of 0.038 – 0.186, while the low category consists of the Sub-Districts of Jatitujuh, Ligung, Jatiwangi, Kadipaten, Bantarujeg, and Talaga. Kertajati, Panyingkiran, Majalengka, Cigasong, Sindang, Rajagaluh, Sigwangi, and Cingambul Sub-Districts belong to the enough category, while the strong category, namely Kasokandel, Argapura, Lemahsugih, and Cikijing Sub-Districts, has a coefficient value close to 1, which is between 0.639 – 0.735.

The negative correlation means that the increase in temperature is not in the same direction as the increase in maize production in the sub-district. The very low category includes the Sub-Districts of Dawuan, Maja, and Malausma with coefficient values between 0.033 – 0.192, while Leuwimunding is a sub-district with a low category which has a coefficient value of 0.285.



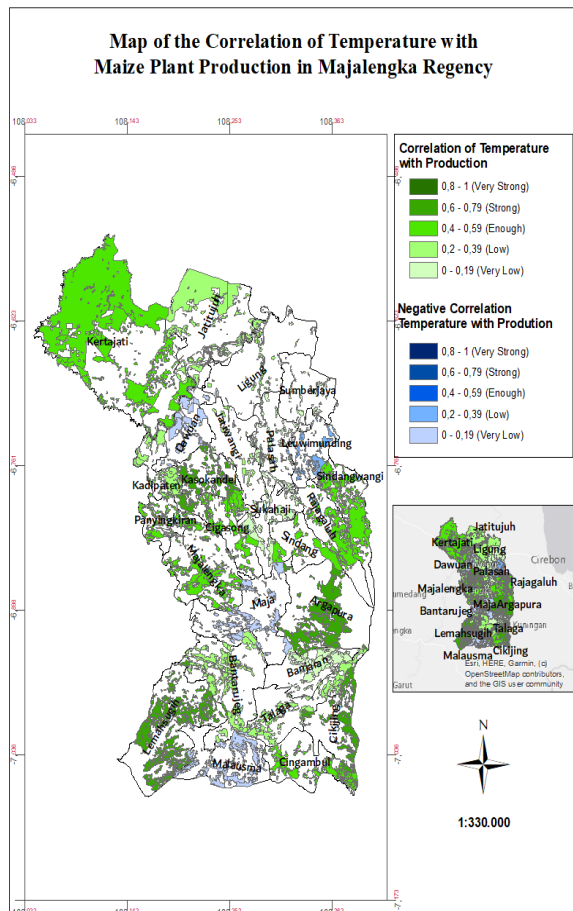


Figure 5. Map of the correlation of temperature with maize production in Majalengka Regency

The results of the correlation analysis in the Figure 6 show a positive correlation that dominates, which means that the increase in temperature is in line with the increase in the productivity of maize in the sub-district. The very low category correlation is Sumberjaya Sub-District with a coefficient value of 0.105, while low correlation includes Jatitujuh, Dawuan, Kadipaten, Panyingkiran, Majalengka, Cigasong, Sukahaji, Sindangwangi, Talaga, Cingambul, and Cikijing Sub-Districts. Kertajati, Ligung, Jatiwangi, Kasokandel, Rajagaluh, Sindang, Maja, Argapura, Banjaran, Bantarujeg, Lemahsugih, and Malausma Sub-Districts are in the enough correlation category with a coefficient value of 0.400 - 0.482. The negative correlation that indicates the increase in temperature is not in line with the productivity of maize in Palasah and Leuwimunding Sub-Districts with coefficient values of 0.390 and 0.220.

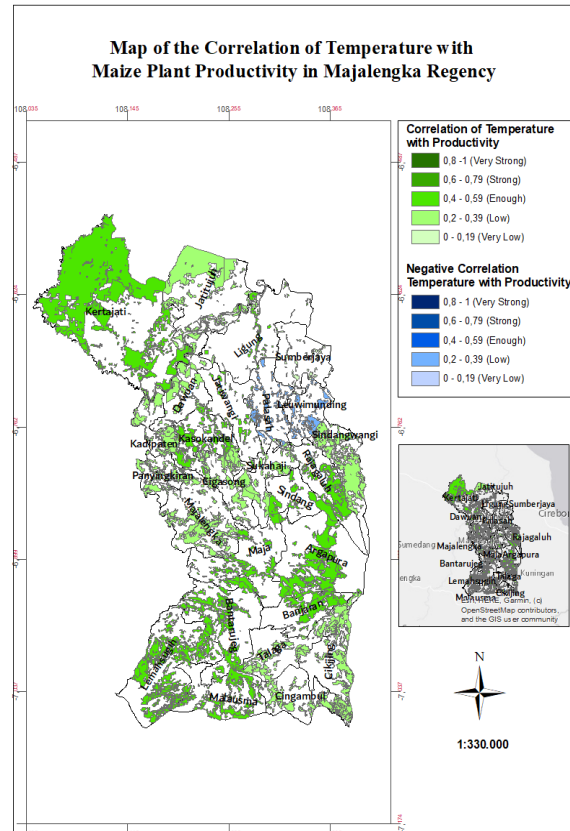


Figure 6. Map of the correlation of temperature with maize productivity in Majalengka Regency

## Conclusion

Majalengka Regency has experienced climate change as indicated by an increase in average air temperature of 0.33 °C which is characterized by an increasing trend and a decrease in rainfall of 56.4 mm which is indicated by a decreasing trend. Based on the results of the correlation analysis, changes in maize production and productivity in Majalengka Regency are significantly correlated with temperature changes, while not significantly correlated with changes in rainfall. Efforts that can be implemented to deal with climate change are the use of superior seeds from high-yielding and early maturing hybrid varieties, managing planting time, building reservoirs, and reforestation as a form of reducing GHS emissions.

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## Growth and yield response of Watani Wado job's tears (*Coix lacryma-jobi* L.) to the application frequency of phosphate fertilizer and paclobutrazol in different growing seasons

**Abstract.** Job's tears (*Coix lacryma-jobi* L.) is a carbohydrate source functional food crop that can be developed as a substitute for rice. This study aims to determine the best application frequency of phosphate (P) fertilizer and paclobutrazol for supporting the growth and yield of job's tears in both rainy and dry seasons. The research was conducted from November 2021 - November 2022 at the Ciparanje research station and Laboratory Centre Universitas Padjadjaran, Jatinangor, Sumedang. The experiment used a randomized block design (RBD) consisting of P fertilizer application frequency (once, twice, and thrice) and paclobutrazol application frequency (once, twice, and thrice) and three replications. The best season was determined using the T-test. The results showed that there was no interaction between growth components and job's tears yield in both rainy and dry season planting. P fertilizer application had a significant effect on lateral branch per tiller in the rainy season and root-shoot ratio in the dry season. Paclobutrazol application had a significant effect on the number of tillers per plant and number of seeds per plant in the rainy season, and the lateral branch per tiller in the dry season. Planting in the rainy season had the best growth and yield components compared to the dry season.

**Keywords:** Dry season · Job's Tears · P Fertilizer · Paclobutrazol · Rainy season

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## Introduction

Job's tears (*Coix lacryma-jobi* L.) is a crop that can be developed for the substitution of rice from paddy as a food source of carbohydrates (Nurmala et al., 2018). Besides being a substitute for food crops, a variety of processed foods, and industries in several Asian countries, job's tears have other advantages compared to other cereals, including the content of a compound of highly flavonoid and glycosides which useful for health (Nurmala, 2011). Job's tears is also a source component bio-actives that have activity as an antioxidant, anticancer, hypolipidemic, and anti-inflammatory (Xi et al., 2016). Therefore, the seeds of job's tears can be used as material food to cope with diabetes, kidney, liver, and lung cancer.

One of the extensively cultivated varieties of Job's tears in Indonesia is mayuen. The mayuen variety has a soft seed shell and is commonly used as cereals (Handayani et al., 2019). One of the local cultivars of the mayuen variety is Watani Wado, which is developed in Sumedang. This cultivar has a significant advantage since it has a high tolerance to drought. Drought can often affect the plant's ability to perform photosynthesis, leading to grain-filling problems. Therefore, this cultivar is highly suitable for dry seasons (Wicaksono et al., 2022). The yield of a job's tears reported fluctuates depending on the season and technique of cultivation. As reported by Ruminta et al., (2018), the rainy season gave a higher yield compared to the dry season.

Job's tears are an indeterminate plant (Nurmala, 2011). This plant has high vegetative growth but low generative ones, so it affects the harvest index (Septian et al., 2023). Job's tears required intensive cultivation techniques to maximize growth and increase reproductive development. One way to increase the yield of job's tears is by fertilizing and administering plant growth retardant.

According to Fahmi et al. (2009), P fertilizer plays a role in improving the quality and yield of job's tears plants. Hütsch et al., (2023) reported that paclobutrazol can inhibit plant vegetative growth and maximize the rate of assimilation so that it can increase the harvest index. The expected effect of these two treatments is an interaction. The use of paclobutrazol can inhibit vegetative growth, so

that P fertilizer can be directly absorbed in the generative phase and the need for P fertilizer is lower. As reported by Zulfaniah et al. (2020), giving P fertilizer accompanied by paclobutrazol at the right time and dose can optimize soybean crop yields. This study aims to determine the best application frequency of phosphate (P) fertilizer and paclobutrazol for supporting the growth and yield of job's tears in both rainy and dry seasons.

## Materials and Methods

This research was conducted from November 2021 at the Experimental Field Faculty of Agriculture and Laboratory Centre of Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java. The altitude of the research location was 750 m above sea level, with the climate type C3 according to Oldman's classification. Analysis of nutrient content carried out in the Laboratory Centre of Universitas Padjadjaran.

The tools that were used for this research included a meter gauge, analytical scale with 0.01-gram accuracy, writing tools, oven, Kjeldahl flask, boiling flask, Soxhlet's tools, and exicator. and a camera. The materials that were used included the seeds of job's tears var. mayuen, NPK 16-16-16 fertilizer, SP-36 fertilizer (36% P), plant growth regulator of paclobutrazol, and Profenos insecticide.

This research was carried out using the method randomized block design two factorial on two season planting consisting of 9 treatments. The first factor is the frequency of P fertilizer application (p1=once of 240 g, p2=twice of 120 g, and p3=thrice of 80 g) with the number of the same dose of 200 kg/ha on 12, 14, and 16 weeks after planting (WAP). The second factor is the frequency of paclobutrazol application (z1=once, z2=twice, and z3=thrice) with a dose of 2000 ppm on 13, 15, and 17 WAP. All treatments were repeated three times, so there were 27 plots. Concerning analysis of characteristic chemistry, there was a method of augmented design (without design trial).

The plots were 4.5 m width and 5 m length. Plant spacing was 60 cm x 40cm. Experimental plots were fertilized with manure at a dose of 2 tons/ha a week before planting. Well-sprouted and healthy job's tears were planted in a hole with 3 cm depth and covered

by soil. The application of NPK according to treatment was given gradually over two times at 4 and 6 WAP by side dressing. P fertilizer treatment was given gradually over three times at 12, 14, and 16 WAP. Paclobutrazol treatment was applied to the plant gradually over three times at 13 (early flowering stage), 15, and 17 WAP. Plant cultivation included watering, weeding, pest, and disease control. Job's tears are harvested 165 days after planting (DAP), or when the seed reaches physiological maturity. Dry and yellowish leaves on the plant physically suggested that seeds were ready to be harvested.

Parameters observed in this experiment are plant height, number of tillers per plant, leaf area index, root shoot ratio, lateral branch per tiller, 100 grain weight, and harvest index. Plant height was measured from the level of ground surface to the tip of main stem using roll meter at 80% flowering before harvest. The number of tillers per plant was recorded by counting the tillers at 13, 15, and 17 WAP (reproductive stage). Leaf area was calculated using the regression equation method for job's tears  $y = 0.227 + 0.683 (\text{width} \times \text{length})$ , ( $R^2 = 94.5\%$ ) at vegetative stage. Root shoot ratio obtained by comparing the dry weight of shoot with the dry weight root plant after harvest. 100 grain weight was measured after harvest using an analytical scale. The harvest index is calculated using the Sitompul and Guritno (1995) formula, which is the crop yield (seeds) divided by the total dry weight of the plant.

Data collected were subjected to analysis of variance (ANOVA) procedures for randomized block design factorial and where treatment means were significant, they were separated using Duncan's test at 5% level of probability using SmartstatXL statistical software.

## Result and Discussion

**Plant height.** The result of statistical data analysis in Table 1 showed that there was no interaction between the application frequency of P fertilizer and paclobutrazol on plant height at 13 WAP, 15 WAP, and 17 WAP. Single effect application frequency of P fertilizer and paclobutrazol did not affect the plant height of job's tears in both rainy and drought season.

There was no significant difference in plant height after the application frequency of P

fertilizer and paclobutrazol. This phenomenon appears to be attributed to genetic factors. Plant growth is influenced by two main factors: genetic and environmental factors (Mahdiannoor & Istiqomah, 2015). The specified environmental factors, namely paclobutrazol and phosphorus fertilizer, did not exhibit a discernible influence on plant height in each treatment. Consequently, genetic factors are deemed more dominant in shaping plant height.

**Table 1. Single effect of different frequency of P fertilizer and paclobutrazol on plant height in rainy and dry seasons**

Treatment	Plant height (cm)		
	Rainy Season*		
	13 WAP	15 WAP	17 WAP
p1	209.46 a	258.70 a	273.17 a
p2	203.06 a	249.44 a	269.54 a
p3	201.58 a	247.14 a	270.39 a
z1	206.99 a	255.94 a	275.63 a
z2	202.09 a	244.61 a	268.03 a
z3	200.68 a	254.73 a	269.43 a
Treatment	Dry Season		
	13 WAP	15 WAP	17 WAP
	13 WAP	15 WAP	17 WAP
p1	202.51 a	205.42 a	209.58 a
p2	200.82 a	203.80 a	209.53 a
p3	204.27 a	206.27 a	214.96 a
z1	204.8 a	206.18 a	213.27 a
z2	204.49 a	206.42 a	212.49 a
z3	201.31 a	202.89 a	208.31 a

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). Paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**Number of tillers per plant.** The interaction between application frequency of P fertilizer and paclobutrazol had not a significant effect on numbers of tillers per plant (Table 2). Single effect of application frequency of P fertilizer and paclobutrazol also did not affect the number of tillers per plant, except for the treatment of paclobutrazol application frequency in rainy season.

Our hypothesis suggests that at the commencement of the growth phase, there was an ample supply of water, a balanced hormonal state, and sufficient nutrients, leading to a relatively uniform number of tillers per plant. Tiller formation is influenced by the content of



N, P, K, and Fe. Low levels of these nutrients result in reduced tiller production. Unlike P, N has a significant impact on the number of tillers, with its availability being the determining factor. Fulfilling the N content in the soil is crucial for achieving optimal tiller production (Ruminta et al., 2017). The application of paclobutrazol may be less targeted in each treatment due to the heat of the sun, causing the sprayed paclobutrazol to evaporate. According to Simanjuntak et al., (2013), improper timing and application methods resulted in paclobutrazol being less effective, thereby not affecting plant growth.

**Table 2. Single effect of different frequency of P fertilizer and paclobutrazol on the number of tillers per plant in the rainy and dry seasons**

Treatment	Number of tillers per plant		
	Rainy Season *		
	13 WAP	15 WAP	17 WAP
p1	6.89 a	8.02 a	8.24 a
p2	7.8 a	7.84 a	8.29 a
p3	7.11 a	7.18 a	7.4 a
z1	6.58 a	7.16 a	7.38 b
z2	6.89 a	7.33 a	7.42 b
z3	6.83 a	8.56 a	9.13 a
Treatment	Dry Season		
	13 WAP	15 WAP	17 WAP
	13 WAP	15 WAP	17 WAP
p1	5.71 a	5.82 a	5.6 a
p2	5.09 a	5.8 a	5.31 a
p3	5.38 a	5.09 a	5.16 a
z1	5.4 a	5.58 a	5.11 a
z2	5.31 a	5.56 a	5.4 a
z3	5.47 a	5.58 a	5.56 a

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). Paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**aLeaf Area Index.** Leaf area index (LAI) is an important indicator reflecting the growth status of plant. Based on the result of the statistical data analysis in Table 3, showed that there was no interaction between the application frequency of P fertilizer and paclobutrazol on leaf area index. Single effect of application frequency of P fertilizer and paclobutrazol did not affect the leaf area index of job's tears. There was no significant difference in leaf area index after the application frequency of P and paclobutrazol, suggesting that the nutrient needs

of the leaves were met while the concentration of paclobutrazol used was not appropriate.

Leaves as photosynthetic organs show that they are affected by P fertilization. The adequate of P will increase nitrogenase activity, and leaf photosynthesis, which increases the growth of plant leaf area and plants can produce more dry matter (Cha et al., 2023). Paclobutrazol significantly reduced plant height and leaf area but increased the leaf area index. Reduced photosynthetic rates were recorded with the treated plants as compared to the control plants (Roseli et al., 2012). The optimal range of leaf area index values for crop plants is 3-5 (Ruminta et al., 2019). In the rainy season planting, the average leaf area index in each treatment was above 3. Therefore, the leaf area index in this experiment was quite good.

**Table 3. Single effect of different frequency of P fertilizer and paclobutrazol on leaf area index, root-shoot ratio, and number of shoots per tiller in job's tears planting in rainy and dry seasons**

Treatment	Rainy		
	Leaf Area Index*	Root -shoot Ratio	Lateral Branch per Tiller*
	Leaf Area Index*	Root -shoot Ratio	Lateral Branch per Tiller*
p1	3.56 a	3.72 a	7.93 a
p2	3.69 a	3.8 a	8.07 a
p3	3.71 a	3.8 a	7.12 b
z1	3.64 a	3.86 a	7.81 a
z2	3.73 a	3.82 a	7.62 a
z3	3.58 a	3.63 a	7.68 a
Treatment	Dry Season		
	Leaf Area Index	Root -shoot Ratio	Lateral Branch per Tiller
	Leaf Area Index	Root -shoot Ratio	Lateral Branch per Tiller
p1	2.96 a	3.39 b	6.53 a
p2	2.91 a	3.86 a	6.6 a
p3	3.17 a	3.56 ab	6.46 a
z1	2.99 a	3.47 a	6.86 a
z2	2.99 a	3.68 a	6.53 ab
z3	3.05 a	3.66 a	6.2 b

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). Paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**Root-shoot Ratio.** Table 3 showed that there was no interaction between the application frequency of P fertilizer and paclobutrazol on root-shoot ratio. Single effect of application frequency of P fertilizer and paclobutrazol also



did not affect root-shoot ratio of job's tears. In the single treatment of P fertilizer application frequency, there was an effect on the root-shoot ratio in the dry season. Irwan et al. (2017), state that the recommended root-shoot ratio for cereal crops is 3. In this research, the root-shoot ratio that is higher than 3 is thought to be due to P fertilizer application and genetic response.

According to Lusiana (2020), a high uptake of P will increase the root-shoot ratio since P plays a crucial role in the formation of plant tissues such as nucleic acids and phospholipids. The positive impact on the above-ground portion of the plant, including leaf area and photosynthetic capacity per unit of leaf area, is influenced by the optimal availability of N and P (Luo et al., 2016). In addition to P fertilizer, paclobutrazol had no impact on root-shoot ratio. We assumed that genetic factors influence the result.

Root-shoot ratio in different growing seasons did not show different results. It is possible that the water factor does not affect the root-shoot ratio, because job's tears a plant that have high tolerance to environmental conditions. Therefore, the root-shoot ratio of job's tears can remain relatively stable or insignificant in variations in the growing season (Xi et al., 2016). Root-shoot ratio is also influenced by several factors such as plant variety, soil conditions, moisture, temperature, and other growth factors.

**Lateral branch per tiller.** The lateral branch per tiller did not exhibit any interaction after treatment. This can be attributed to the influence of plant genetic factors and environmental conditions. No significant differences were observed among single factors, except for the frequency of P fertilizer application in the rainy season and paclobutrazol application in the dry season. Environmental factors such as temperature, humidity, and soil conditions may influence the interaction between P fertilizer and paclobutrazol. If these conditions are not optimal for the absorption and influence of these substances on plants, the interaction may not be effective (Kamran et al., 2018).

The frequency of P fertilizer application during the rainy season had a significant impact on the lateral branches per tiller. The number was higher when P fertilizer was applied twice compared to other treatments. However, in the dry season, the frequency of P fertilizer application did not have a significant effect. This

could be attributed to water scarcity, which inhibits the growth and formation of shoots in job's tears plants. Water scarcity can disrupt cellular functions and negatively affect growth. During the rainy season, a single treatment of paclobutrazol did not significantly impact the lateral branches per tiller, but it had a significant effect during the dry season. The most effective method was to use paclobutrazol once, resulting in 6.86 lateral branch per tiller. In the rainy season, plants experience favorable environmental conditions, such as warmer temperatures, high humidity, and sufficient rainfall, which often lead to active growth and high levels of natural hormone production. Therefore, the use of paclobutrazol, which inhibits plant growth, may not have a significant effect in such conditions (Desta & Amare, 2021).

**Table 4. Single effect of different frequency of P fertilizer and paclobutrazol on number spike per tillers**

Treatment	Number of Spike per Tiller	
	Rainy* Season	Dry Season
p1	27.56 a	27.32 a
p2	31.04 a	25.53 a
p3	28.6 a	25.07 a
z1	28.58 a	27.99 a
z2	29.04 a	24.39 a
z3	29.58 a	25.54 a

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). Paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**Number of Spikes per Tiller.** Statistical analysis showed that there was no interaction between the application frequency of P fertilizer and paclobutrazol on number of spikes per tillers. The single effect of the application frequency of P fertilizer and paclobutrazol is presented in Table 4. The single application of P fertilizer did not show a significant effect on the number of spikes per tiller per plant during both rainy and dry seasons. Even during the dry season, P fertilizer may not dissolve due to the lack of water. This is due to the low rainfall, which caused the P fertilizer to not dissolve and be absorbed by the plants. Even during the dry season, P fertilizer may not dissolve due to the

lack of water. According to Acquaaah (2005) cited in Ruminta et al. (2018), water plays a crucial role in soil as a solvent for further nutrients required for plant growth.

Treatment with paclobutrazol did not affect the number of spikes per tiller, regardless of whether the plants were grown in rainy or dry seasons (Table 4). The plant's ability to produce spikes is determined by the number of spikes and the groundwater status during the vegetative period, as well as the original photosynthate used for vegetative growth, which will later be utilized for generative growth (Watson, 2006). The availability of sufficient water during the vegetative phase affects relative shoot growth, resulting in a similar appearance (Nurmala et al., 2018).

**Table 5. Single effect of different frequency of P fertilizer and paclobutrazol on number of seeds per plant**

Treatment	Number of Seeds per Plant	
	Rainy* Season	Dry Season
p1	678.6 a	403.59 a
p2	744.94 a	357.09 a
p3	703.94 a	353.54 a
z1	648.86 b	379.85 a
z2	689.78 ab	369.98 a
z3	788.84 a	364.4 a

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). Paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**Number of Seeds per Plant.** There was no interaction effect between the application frequency of P fertilizer and paclobutrazol on the number of seeds per plant. Single effect application frequency of P fertilizer and paclobutrazol did not affect the number of seeds per plant job's tears on rain season and drought season (Table 5).

The addition of nutrients in this study did not show any significant differences in the character number of seeds per plant. We suspected that the presence of phosphorus is very crucial, and it required in large quantities to support grain formation. Phosphorus is a macro element required by plants for energy transfer, signal transduction, and enzyme activation

(Wang et al., 2017). In cereal crops like wheat, a limited supply of phosphorus can decrease grain yields by restricting the number of productive tillers (El Mazlouzi et al., 2020). Climatic factors such as solar radiation, temperature, and rainfall significantly influence the germination and growth of job's tears seeds.

In addition to phosphorus fertilization, paclobutrazol did not exhibit a significant effect on the number of seed per plant, except during the rainy season. In the rainy season planting, the application of paclobutrazol three times resulted in the highest seed count per plant, reaching 788.84. This finding is consistent with the report by Xia et al., (2018), suggesting that the use of paclobutrazol during the rainy season, when vegetative plant growth tends to increase, can regulate growth, and facilitate more efficient allocation of plant resources and energy towards flower and seed formation.

**Table 6. Single effect of different frequency of P fertilizer and paclobutrazol on 100 grains weight and harvest index**

Treatment	Weight of 100 Grains		Harvest Index	
	Rainy*	Dry	Rainy*	Dry
p1	10.5 a	10.18 a	0.27 b	0.32 a
p2	10.79 a	9.73 a	0.29 b	0.32 a
p3	10.44 a	10.11 a	0.37 a	0.33 a
z1	10.31 a	9.91 a	0.29 a	0.29 a
z2	10.89 a	9.97 a	0.33 a	0.35 a
z3	10.53 a	10.14 a	0.31 a	0.33 a

Description: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at 5% level. \*=showing significant differences among seasons based on a T-test at a 5% level of significance. P fertilizer application frequency (p1=once, p2=twice, and p3=thrice). paclobutrazol application frequency (z1=once, z2=twice, and z3=thrice).

**Weight of 100 grains.** The weight of 100 grains can be determined by weighing them using an analytical scale. Table 6 showed that there was no interaction between the application frequency of P fertilizer and paclobutrazol on weight of 100 grains. The weight of 100 grains was not significantly affected by the frequency of single P fertilizer application, in both the rainy and dry seasons. Phosphorus plays an important role in fruit and seed formation, and according to (Sitepu et al., 2015), the application of phosphorus fertilizer leads to differences in grain weight by increasing the availability of

sufficient assimilation, which leads to the accumulation of more stored food in the grains.

In addition to fertilization, paclobutrazol did not significantly affect the weight of 100 grains. It is hypothesized that abscisic acid and ethylene play a more crucial role in seed production compared to gibberellin. Therefore, paclobutrazol does not have a significant impact. However, Nurmala et al., (2018) reported that applying paclobutrazol at a concentration of 1000-2000 ppm can improve several growth and yield parameters in job's tears plants, including leaf area index, number of productive tillers, weight of 100 grains, harvest index, broken rice yield, and seed hardness.

**Harvest Index.** The harvest index is a comparison between the weight of economically valuable plant parts (seeds) and the total weight of the whole plant. Table 4 shows that the frequency of P fertilizer application has a significant effect on the yield index during the rainy season, but not during the dry season. This is likely due to the dry season causing insufficient water supply for plants and hindering the complete dissolution of P fertilizer. Applying P fertilizer three times during the rainy season leads to a higher harvest index compared to other frequencies. A high harvest index indicates efficient photosynthesis in plants, allowing for the translocation of nutrients to the organs intended for harvest (Ruminta et al., 2017). Phosphorus plays a crucial role in directing the outcomes of photosynthesis toward the generative organs of the plant, enhancing the harvest index (Irwan et al., 2017).

There was no significant effect on harvest index from the application of paclobutrazol. This lack of effect may be due to genetic factors, environmental influences, or improper application methods. As noted by Simanjuntak et al. (2013), incorrect timing and application methods can reduce the effectiveness of paclobutrazol. To ensure the effective application and impact of paclobutrazol, it is important to consider the plant's growth stage, weather conditions, and temperature, as noted by Simanjuntak et al. (2013).

## Conclusion

1. There was no interaction between the application frequency of P fertilizer and

paclobutrazol on the growth components and yield of Job's tears during both rainy and dry season plantings.

2. The twice P fertilizer application treatment significantly affect the number of lateral branches per tiller in rainy season and root-shoot ratio Job's tears in dry season. The thrice P fertilizer application significantly affect the harvest index of Job's tears in the rainy season. The thrice paclobutrazol application had a significant effect on the number of tillers per plant at the age of 17 WAP, the number of seeds per plant when planting in the rainy season, and the number of lateral branches per tiller when planting in the dry season.
3. Planting job's tears in rainy season gave higher growth and yield performance rather than the dry season, except the root-shoot ratio character.

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## The effectiveness of different formulations of glyphosate herbicides under simulated rainfall conditions in controlling various weeds

**Abstract.** Herbicide effectiveness is affected by herbicide formulation, rainfall, and weed type. Differences in glyphosate salt formulations and 2,4 D amine mixtures may result in variations in the herbicide's ability to wash off the herbicide due to rain. This study aimed to assess the effectiveness of different glyphosate herbicide formulations under varying rainfall in controlling various weeds. The experiment took place in a controlled greenhouse environment, utilizing a split-plot experimental design with four replications. The main plot was assigned to six different rainfall timings: 0 hours after application (HAA), 1 HAA, 2 HAA, 3 HAA, 4 HAA, and no rainfall. The subplots involved different herbicide formulations, namely isopropylamine glyphosate (h1), potassium glyphosate (h2), sodium glyphosate (h3) and glyphosate herbicide, IPA herbicide glyphosate + 2,4 D Amine (h4) and without any herbicide application (h0). Various parameters were observed, including weed dry weight and percentage of mortality growth. The results showed that the effectiveness of each herbicide formulation was different among weed species under simulated rainfall conditions. *Ageratum conyzoides* could be controlled using isopropylamine glyphosate and potassium glyphosate with rainfall at 1 HAA. *Axonopus compressus* could be controlled by isopropylamine glyphosate and sodium glyphosate with rainfall at 1 HAA, while *Borreria alata* and *Cyperus rotundus* were effectively controlled by isopropylamine glyphosate with rainfall at 1 HAA.

**Keywords:** 2,4 D amine · Formulations · Glyphosate · Rainfall · Weed

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## Introduction

A weed is a plant that grows in an area where it is seen as unwanted by humans because it interferes with their requirements, ambitions, or preferences (Borah, 2023). Weeds are a significant biological element in crop production that reduces yield, accounting for about 45% of crop yield loss (Korav et al., 2018). Weeds have the potential to reduce agricultural yield and quality of production by competing with crops for nutrients including water, light, fertilizer, and growing space (Khan et al., 2021).

Losses due to weeds can be reduced with proper weed control. Herbicide application is the most practical, effective, and economical way to reduce weed problems, crop losses, and production costs (Latiff et al., 2009). Glyphosate herbicide is the most widely used and popular herbicide in the world (Baylis, 2000; Singh et al., 2011). Glyphosate is a non-selective systemic herbicide that has a broad spectrum. Glyphosate herbicide is effective against more than 100 broadleaf and grass weeds and more than 60 species of annual weeds (Singh et al., 2011).

Continuous application of glyphosate—sometimes more than twice a year in the same crop—has put the flora under a lot of selection pressure, which has led to the emergence of weeds that are tolerant or resistant to glyphosate (Heap, 2020). Efforts that can be made are by mixing herbicides to broaden the spectrum of weed control, and reducing weed resistance to one herbicide to prevent homogeneous weed vegetation in an area (Sembodo & Wati, 2021). Glyphosate herbicide can be mixed with 2,4 D Amine. Palma-Bautista et al. (2021) stated that glyphosate with 2,4-D in a tank mix is an effective substitute for managing weeds that are resistant to and tolerant to glyphosate.

Apart from that, the active ingredient glyphosate is applied in the form of a salt addition formulation due to its low solubility. The addition of different salts to glyphosate formulations can increase water solubility by up to 90% (Shaner, 2014; Dalazen et al., 2020). Differences in herbicide formulations and concentrations can affect the rate of absorption and translocation of herbicides and the effectiveness of weed control (Martini et al., 2003; Souza et al., 2014). The glyphosate salt formulation used in this experiment is isopropylamine, potassium, and sodium.

Effective herbicide applications require successful deposition, retention, uptake, and translocation of the herbicide onto the plant (Zwertvaegher et al. 2014). Environmental factors such as rain also determine the effectiveness of herbicides. The amount of rainfall and the time of rain significantly impact the effectiveness of the herbicide (Stewart et al., 2012). Unexpected rain causes the herbicide to leach, thereby reducing its effectiveness (Kurniadie et al., 2022). The efficacy of glyphosate herbicides was reduced by 50% after 1 hour of rain after application (Bariuan et al., 1999).

Herbicide effectiveness is also influenced by the characteristics of the target weeds. Various weed species exhibited varying reactions to rainfall, as leaf features impact absorption and hence herbicide efficiency (Rodrigues, 2018). The effectiveness of herbicide absorption is affected by species, growth stage, habitat, morphology, and leaf wax composition of weeds (Costa et al., 2017). This study aims to determine the ability of several glyphosate herbicide formulations with different rainfall times to control various types of weeds.

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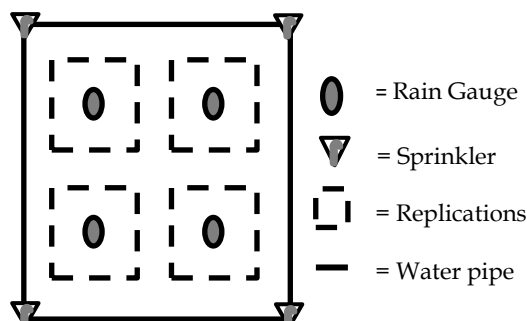
## Materials and Methods

The experiment was carried out at the Experimental Laboratory of the Faculty of Agriculture, Universitas Padjadjaran from January to March 2019. The materials used were *Ageratum conyzoides*, *Borreria alata*, *Axonopus compressus*, *Cyperus rotundus*, herbicide Isopropylamine glyphosate 486 g/L, potassium glyphosate 660 g/L, sodium glyphosate 170 g/L, and mixed herbicide IPA glyphosate 300 g/L + 2,4 D amine 100 g/L with a recommended dose of 1.5 L each /Ha. This study used a split-plot experimental design with four replications. The main plot was assigned to six different rainfall timings: 0 hours after application (HAA), 1 HAA, 2 HAA, 3 HAA, 4 HAA, and no rainfall. The subplots involved different herbicide formulations, namely isopropylamine glyphosate (h1), potassium glyphosate (h2), sodium glyphosate (h3) and glyphosate herbicide, IPA herbicide glyphosate + 2,4 D amine (h4) and without any herbicide application (h0).

Each weed with 2-3 growing leaves was transferred to a 20 cm diameter pot filled with planting medium, and then maintained properly for two weeks. After two weeks, the weeds that had grown were moved into a 4 x 4 m plot



(Figure 1) and each herbicide was applied according to the time it rained. The rainfall used is 10 mm/day (rainfall in the area of PTPN VIII Subang). If the rain gauge used has a diameter of 20 cm, as shown in Figure 1.



**Figure 1. Layout of sprinklers, rain gauges, and location of experimental plots on a rain simulation plot.**

The herbicide was applied using a semi-automatic knapsack sprayer and T-Spray nozzles with a pressure of 15-20 psi and a volume of 400 l/ha. The rain simulation was carried out according to the rain time treatment after herbicide application. Observation of dry weight is done by weed harvesting at 3 weeks after application (WAA). The weeds from each treatment were taken and cleaned from the soil, then added to the weeds and heated in the oven until they reached a constant weight at 80°C. After baking, weeds were weighed for dry weight. The percentage of weed damage is calculated by the following formula (Chuah et al., 2004):

$$\% \text{ Damage} = \left( 1 - \left( \frac{\text{dry weight of treatment}}{\text{dry weight of control}} \right) \right) \times 100$$

The results obtained were analyzed by analysis of variance with the F test and the average treatment used Scott Knott's advanced test at a significant level of 5%. The original dry weight data were transformed by root transformation ( $x+0.5$ ) and the original data on weed damage percentage were transformed by root arc sin transformation ( $\%+0.5$ ).

## Results and Discussion

The target weeds tested in this study were broadleaf: *Ageratum conyzoides*, and *Borreria alata*, grass weed: *Axonopus compressus*, sedge weed: *Cyperus rotundus* that is the dominant weed in the

oil palm plantation area of PTPN VIII Subang. *Borreria alata*, *Ageratum conyzoides*, *Asystasia intrusa*, *Imperata cylindrica*, *Paspalum conjugatum*, *Setaria plicata*, *Emilia sonchifolia*, and *Erigeron sumantresis* including weeds that are almost always present or dominant in oil palm plantations (Purwasih et al., 2013).

**Weed dry weight and percentage *Ageratum conyzoides*.** Based on observations of dry weight and percentage of weed damage in *Ageratum conyzoides* the herbicides isopropylamine and potassium glyphosate with a raining time of 1 HAA had a lower dry weight and a higher percentage of damage compared to other treatments with the same raining time and relatively the same as the treatment without rain. This shows that the application of isopropylamine and potassium glyphosate herbicides with 1 HAA rainfall time has been able to control weeds *Ageratum conyzoides* (Table 1). The same results were also shown by the research of Kurniadie & Umiyati (2019) that the herbicide potassium glyphosate was able to control weeds *Ageratum conyzoides* with a rain time of 0-1 hour after application. The effectiveness of isopropylamine glyphosate and potassium glyphosate in controlling *Ageratum conyzoides* can be due to their good absorption. Increasing the absorption ratio is one of the keys to improving herbicide efficacy, according to Li et al. (2019).

Sodium glyphosate and IPA glyphosate+2,4 D amine with a raining time of 3 HAA showed dry weight and percentage of weed damage in *Ageratum conyzoides* same as with the no rain treatments. *Ageratum conyzoides* are effectively controlled by the sodium glyphosate and IPA glyphosate+2,4 D amine after three hours without rain. Research by Pedrinho Junior et al. (2002) stated that EPSPS-inhibiting herbicide formulations were effective in controlling weeds by simulating rain for up to four hours after application. Herbicide 2,4 D amine without rain for 30 minutes is still effective in controlling *Senna obtusifolia* (Souza et al., 2014).

**Weed dry weight and percentage of *Borreria alata* weed damage.** Table 2 shows that isopropylamine glyphosate with 1 HAA raining time has been able to control *Borreria alata* which was indicated by a lower dry weight and a higher percentage of damage than the other treatments and relatively the same as the treatment without rain. The effectiveness of glyphosate herbicides is not affected by rainwater washing within 2 hours after application (Girsang, 2005).

**Table 1. The effect of various herbicide formulations with differences rainfall times on weed dry weight and percentage of *Ageratum conyzoides* weed damage**

Time occurrence of rain	Dry weight (g)					Percentage of weed damage (%)			
	Herbicide formulation					Herbicide formulation			
	h0	h1	h2	h3	h4	h1	h2	h3	h4
0 HAA	2.58 a A	0.73 a B	0.85 a B	1.93 a A	2.38 a A	75.12 b A	69.23 b A	39.70 c B	24.62 d B
1 HAA	2.63 a A	0.08 b C	0.23 b C	0.73 b B	1.23 b B	96.43 a A	91.43 a A	75.49 b B	66.35 c B
2 HAA	2.33 a A	0.00 b C	0.00 b C	0.40 b B	0.45 c B	100.0 a A	100.0 a A	88.32 b B	87.44 b B
3 HAA	2.45 a A	0.00 b B	0.00 b B	0.13 c B	0.00 d B	100.0 a A	100.0 a A	95.12 a A	100.0 a A
4 HAA	2.10 a A	0.00 b B	0.00 b B	0.00 c B	0.00 d B	100.0 a A	100.0 a A	100.0 a A	100.0 a A
No rainfall	3.25 a A	0.00 b B	0.00 b B	0.00 c B	0.00 d B	100.0 a A	100.0 a A	100.0 a A	100.0 a A

Note: The average number followed by the same lowercase letter in the column (type of herbicide) and uppercase letter in the row (time of rain) is not significantly different based on the Scott-Knott test at the 5% level. HAA: hours after application, h0: without herbicide, h1: isopropylamine glyphosate, h2: potassium glyphosate, h3: sodium glyphosate, h4: IPA glyphosate +2.4 D amine

**Table 2. The effect of various herbicide formulations with different rainfall times on weed dry weight and percentage of *Borreria alata* weed damage**

Time occurrence of rain	Dry weight (g)					Percentage of weed damage (%)			
	Herbicide formulation					Herbicide formulation			
	h0	h1	h2	h3	h4	h1	h2	h3	h4
0 HAA	2.70 a A	0.53 a C	1.15 a B	1.43 a B	1.43 a B	82.44 b A	59.73 b B	50.32 c B	52.21 c B
1 HAA	2.10 a A	0.00 b C	0.75 a B	1.00 a B	1.25 a B	100.0 a A	76.13 b B	60.34 c B	60.78 c B
2 HAA	2.20 a A	0.00 b C	0.00 b C	0.33 b C	0.63 b B	100.0 a A	100.0 a A	90.55 b B	77.07 b B
3 HAA	2.48 a A	0.00 b B	0.00 b B	0.00 b B	0.00 c B	100.0 a A	100.0 a A	100.0 a A	100.0 a A
4 HAA	2.75 a A	0.00 b B	0.00 b B	0.00 b B	0.00 c B	100.0 a A	100.0 a A	100.0 a A	100.0 a A
No rainfall	3.08 a A	0.00 b B	0.00 b B	0.00 b B	0.00 c B	100.0 a A	100.0 a A	100.0 a A	100.0 a A

Note: The average number followed by the same lowercase letter in the column (type of herbicide) and uppercase letter in the row (time of rain) is not significantly different based on the Scott-knott test at the 5% level. HAA: hours after application, h0: without herbicide, h1: isopropylamine glyphosate, h2: potassium glyphosate, h3: sodium glyphosate, h4: IPA glyphosate +2.4 D amine

Potassium glyphosate herbicide can control *Borreria alata* with a rain-free time of up to 2 hours after application. *Borreria alata* can be controlled by the herbicide sodium glyphosate and IPA glyphosate + 2.4 D if there is no rain up to 3 hours after application. Kurniadie & Umiyati (2019) reported that potassium glyphosate herbicide is effective in controlling *Borreria alata* with rain time 2 hours after application.

**Weed Dry Weight of *Axonopus compressus*.** Results of observations of weed dry weight *Axonopus compressus* showed that isopropylamine and sodium glyphosate herbicides with 1 HAA raining time were able to control weeds *Axonopus compressus* which is shown with low dry weight and does not differ from the treatment without rain (Table 3). Rain that occurs 15 minutes – 6 hours after herbicide application can reduce the efficacy of glyphosate herbicides (Boerboom, 2006).

**Table 3. The effect of various herbicide formulations with different rainfall times on dry weight of *Axonopus compressus***

Time occurrence of rain	Herbicide formulation				
	h0	h1	h2	h3	h4
0 HAA	3.65 a A	1.90 a B	1.33 a B	1.68 a B	2.20 a B
1 HAA	3.48 a A	0.63 b B	0.68 a B	0.70 b B	1.10 a B
2 HAA	4.03 a A	1.18 a B	0.50 a B	1.30 a B	1.73 a B
3 HAA	3.85 a A	0.00 b C	0.88 a B	1.63 a B	1.20 a B
4 HAA	3.85 a A	0.00 b B	0.00 b B	0.55 b B	0.95 a B
No rainfall	5.03 a A	0.00 b B	0.00 b B	0.00 b B	0.00 b B

Note: The average number followed by the same lowercase letter in the column (type of herbicide) and uppercase letter in the row (time of rain) is not significantly different based on the Scott-Knott test at the 5% level. HAA: hours after application, h0: without herbicide, h1: isopropylamine glyphosate, h2: potassium glyphosate, h3: sodium glyphosate, h4: IPA glyphosate +2.4 D amine

**Weed Damage Percentage of *Axonopus compressus*.** Table 4 shows that the results of observations on the percentage of damage show the independent effect of the herbicide formulation and the time it rains. The herbicides isopropylamine glyphosate and potassium glyphosate showed a higher percentage of damage compared to the sodium glyphosate and IPA glyphosate+2.4 D amine treatments, while the 4 HAA treatment had a high percentage and was relatively the same as the no-rain treatment. Potassium glyphosate herbicide that was rained 0 – 4 hours after application was not effective in controlling growth of *Asystasia intrusa* weed (Priambodo, 2017).

**Dry Weight and Percentage of *Cyperus rotundus* Weed Damage.** Table 5 shows the results of observing the dry weight of *Cyperus rotundus* that the herbicides isopropylamine glyphosate, potassium glyphosate, and sodium glyphosate with a raining time of 1 HAA had a dry weight of *Cyperus rotundus* relatively the same as the treatment without rain. Observation of damage percentage showed that the herbicide isopropylamine glyphosate with 1 HAA rain time showed a high percentage of weed damage and was relatively the same as the treatment without

rain. Rain simulation with 1 hour after application reduced the efficacy of glyphosate on *Abutilon of Theophrastus*, *Cyperus esculantus* and *Echinochloa cruss-galli* (Reddy & Singh, 1992).

**Table 4. The effect of various herbicide formulations with different rainfall times on the percentage of *Axonopus compressus* weed damage**

Treatments	Percentage of weed damage (%)
Time occurrence of rain :	
0 hours after application	64.67 c
1 hours after application	85.48 b
2 hours after application	73.76 c
3 hours after application	80.12 b
4 hours after application	92.60 a
No rainfall	100.00 a
Herbicide formulation :	
Isopropylamine glyphosate	87.39 a
Potassium glyphosate	88.22 a
Sodium glyphosate	80.42 b
IPA glyphosate + 2,4 D Amine	75.05 b

Note: The average score followed by the same letter is not significantly different based on the Scott-Knott test at the 5% level.

*Cyperus rotundus* effectively controlled with glyphosate potassium, sodium glyphosate, and IPA glyphosate +2,4 D amine with 2 HAA rain simulation. Souza et al. (2011) revealed that the effectiveness of herbicides is closely related to absorption, the lower the absorption, the lower the effectiveness. In general, herbicide absorption by weeds takes more than 2 hours (Sriyani, 2010).

Each herbicide formulation showed different abilities in controlling various target weeds with different rainfall periods. Differences in the efficacy of glyphosate herbicides in controlling various types of weeds depend on different rain-free periods (Kurniadie & Umiyati, 2019). The rain-free period for herbicide efficacy depends on the chemical nature of the herbicide and the degree of application (Ganon & Yalverton, 2008).

Based on the data on dry weight and damage percentage, it is known that the herbicide isopropylamine glyphosate was able to control all the weeds tested with 1 HAA rainfall. Research on glyphosate salt with rain simulation reported that glyphosate isopropylamine salt formulation controls *Brachiaria decumbens* better than potassium and diammonium (Costa et al.,

2017). Glyphosate formulations that increase acid absorption can increase herbicide efficacy (Travlos et al. 2007). Nalejawa et al. (1996) stated that glyphosate isopropylamine salt showed better absorption than other salts.

Isopropylamine glyphosate, potassium glyphosate, and sodium glyphosate with a raining time of 1 HAA were still effective in controlling some of the target weeds. IPA glyphosate+2,4D Amine herbicide is effective in controlling *Cyperus rotundus* with a rain time of 2 HAA. Research from Souza et al. (2014) reported that glyphosate herbicide formulations (Roundup original, Roundup Transrob, Roundup Transrob R, and Roundup Ultra) treated after 15 minutes were still effective in controlling *Senna obtusifolia*. Weed control is reduced when rainfall occurs soon after post-herbicide treatment because the herbicide is rinsed off the leaves before absorption is complete (Thakur et al., 2018).

The effectiveness of herbicides is affected by the time it rains and the intensity of the rain. Glyphosate herbicides (720 and 1440 g ha<sup>-1</sup>) at *I. purpurea*, and *Euphorbia heterophylla* with six rain cycles after application, showing a decrease in dry weight decreasing as the rain interval increases after application (Monquero & Silva, 2007). Rain that occurs 15 minutes – 6 hours after herbicide application can reduce the efficacy of glyphosate herbicides (Boerboom, 2006). Manik

& Dad's research (2020) shows that a rainfall intensity of 5 mm/hour can reduce the effectiveness of herbicides in controlling *Ageratum conyzoides* and *Cyperus rotundus*.

The resistance of various herbicide formulations to rain is influenced by many factors. The relationship between rainfall, speed, and volume of glyphosate absorbed by plants and the effectiveness of glyphosate herbicides depends on processes such as retention of the herbicide on the leaf surface, leaf penetration, translocation, and inhibition of the EPSPS target enzymes (Costa et al., 2017). Satchivi et al. (2000) revealed that the uptake and translocation of glyphosate is species dependent. Species with high epicuticular wax can inhibit herbicide absorption, while high stomata density can increase herbicide uptake (Costa et al., 2017).

## Conclusion

Isopropylamine glyphosate with a rainfall period of 1 HAA was able to control all the weeds tested, while the herbicide potassium glyphosate with a rainfall period of 1 HAA was able to control *Ageratum conyzoides*. Sodium glyphosate herbicide with 1 HAA rain time had been able to control *Axonopus compressus* while IPA glyphosate herbicide + 2.4 D amine with 2 HAA rain time could control *Cyperus rotundus*.

**Table 5. The effect of various herbicide formulations with differences in the timing of rainfall on weed dry weight and percentage of *Cyperus rotundus* weed damage**

Time occurrence of rain	Dry weight (g)					Percentage of weed damage (%)			
	Herbicide formulation					Herbicide formulation			
	h0	h1	h2	h3	h4	h1	h2	h3	h4
0 HAA	1.00 b A	0.78 a A	0.88 a A	0.80 a A	0.85 a A	38.41 b A	35.00 c A	41.03 c A	35.64 c A
1 HAA	0.93 b A	0.25 b B	0.15 b B	0.23 b B	0.68 a A	75.83 a A	73.04 b A	67.65 b A	66.59 b A
2 HAA	0.90 b A	0.03 b B	0.00 b B	0.00 b B	0.00 b B	97.92 a A	100.0 a A	100.0 a A	100.0 a A
3 HAA	0.85 b A	0.00 b B	0.03 b B	0.03 b B	0.00 b B	100.0 a A	98.53 a A	98.53 a A	100.0 a A
4 HAA	1.03 b A	0.00 b B	0.00 b B	0.03 b B	0.08 b B	100.0 a A	100.0 a A	97.50 a A	100.0 a A
No rainfall	1.40 a A	0.00 b B	0.00 b B	0.00 b B	0.00 b B	100.0 a A	100.0 a A	100.0 a A	100.0 a A

Note: The average number followed by the same lowercase letter in the column (type of herbicide) and uppercase letter in the row (time of rain) is not significantly different based on the Scott-Knott test at the 5% level. HAA: hours after application, h0: without herbicide, h1: isopropylamine glyphosate, h2: potassium glyphosate, h3: sodium glyphosate, h4: IPA glyphosate +2.4 D amine

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Ulinnuha Z · Tini EW · Farid N · Shalatin D

## The effect of tryptone and tomato juice addition on the growth performance of strawberry (*Fragaria ananassa*) explant under *in vitro* condition

**Abstract.** Strawberries are a fruit commodity that has high economic value. The obstacle in strawberry production is the lack of high-quality seed availability with disease-free properties. Tissue culture offers a promising solution to increase both the quantity and quality of strawberry seeds. This study aims to assess the effect of different concentrations of tomato juice and tryptone on the growth of strawberry explants under *in vitro* growing condition. The research was conducted at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, from December 2022 to May 2023. The study employed a Completely Randomized Design (CRD) with two factors. The first factor was tomato juice (Z), consisting of Z0 = 0 mL/L, Z1 = 50 mL/L, Z2 = 100 mL/L. The second factor was tryptone (T), consisting of T0 = 0 g/L, T1 = 1 g/L, T2 = 2 g/L, T3 = 3 g/L. The addition of 50 mL/L tomato juice solely (T0Z1) resulted in the highest plant height, number of leaves, number of branches, and leaf width. Meanwhile, the addition of 2 g/L tryptone solely showed the highest germination rate.

**Keywords:** Disease-free explant · Tomato juice · Tryptone

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## Introduction

Strawberries are a sub-tropical fruit commodity with significant potential for development in Indonesia, particularly in highland areas. Strawberries are rich in phytochemicals, especially phenolic compounds, which offer health benefits (Nofrianinda et al., 2018). The Indonesian Central Statistics Agency reported that strawberry production in Indonesia in 2021 was 9,860 tonnes, marking a decrease from 2017 when it was capable of producing up to 12,225 tonnes. The decrease in strawberry production is partially related to the availability of an adequate supply of disease-free seeds, because the propagation via stolons is the potential transmission of diseases from the parent plant to the daughter plant, such as *Plomobacter* (Dittmer et al., 2021).

Utilizing tissue culture for strawberry propagation can serve as a solution to increase strawberry seed production and produce disease-free seedlings (Mohapatra & Batra, 2017). Tissue culture is a technique that involves isolating plant components like cells, tissues, or organs and cultivating them in a sterile environment to enable these components to reproduce and regenerate into complete plants (Loyola-Vargas & Ochoa-Alejo, 2018). Furthermore, the propagation through tissue culture offer the benefits of requiring minimal storage space, enabling the acceleration of plant growth cycles, and uniform seedling growth by controlled environment (Espinosa-Leal et al., 2018).

The composition of the culture medium utilized depends on the specific plant being propagated. This medium typically includes agar, mineral salts, vitamins, and growth regulators such as plant hormones (Semiarti, 2018). The medium commonly used in strawberry plant culture is Murashige and Skoog (MS) medium, characterized by its high content of inorganic salts. Increasing the regeneration of explants requires the addition of growth regulators to the growth medium. Tomato juice accelerates seed growth because it contains various organic compounds such as carbohydrates, amino acids, and growth hormones (Yunita et al., 2023). The growth regulator present in tomato juice plays a role in chlorophyll formation in plants. The cytokinin content is involved in cell division within

meristem tissue (Dewi et al., 2021). Furthermore, adding tomato extract to the medium can accelerate germination, increase chlorophyll biosynthesis, and suppress embryo death (Muharyati et al., 2016).

The application of amino acids to plants can enhance growth by increasing plant dry weight, chlorophyll content, nitrate reductase activity, and glutamine synthetase enzyme activity (Jalali et al., 2020). Tryptone, as a source of amino acids, has been reported to promote the growth of *Cymbidium* orchids (*Cymbidium kanran* Makino). Tryptone provides a diverse array of nutrients, including nitrogen, carbon, sulfur, and various micronutrients, which can serve as a nutrient source for plants instead of other types of amino acids (Zhu et al., 2019). Additionally, the nitrogen content in tryptone, which is as high as 13.3%, is reported can increase the number of nodes in *Eustoma grandiflorum* flowers (Ohta et al., 2004). This research aimed to evaluate the influence of various concentrations of tomato juice and tryptone on the growth of strawberry explant under *in vitro* condition.

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## Materials and Methods

This research was conducted at the Plant Breeding and Biotechnology Laboratory, Faculty of Agriculture, Jenderal Soedirman University, from December 2022 to May 2023. The materials used in this research included Mencir strawberry seeds, Murashige and Skoog medium, distilled water, tomatoes, tryptone, sucrose, agarose, KOH solution, HCl solution, and 70% alcohol. Additionally, the following equipment were used: Laminar Air Flow Cabinet, tape, plastic wrap, autoclave, analytical balance, pH meter, magnetic stirrer, culture bottle, alcohol bottle, beaker, measuring flask, gas stove, pan, stirrer, micropipette, spatula, tweezers, blade, scalpel, masking, Petri dishes, matches, and Bunsen burner.

Sterilization using 30% Clorox (with 5.25% NaOCl content) soaking for 10 minutes. Then rinsed with sterile distilled water which was repeated 3 times.

This study used a Completely Randomized Design (CRD) with two factors, which were the treatments involving tomato juice and tryptone. The first factor, i.e., tomato juice, consisted of three levels: Z0 (control or no tomato juice), Z1 (50 mL/L), and Z2 (100 mL/L), while the second

factor, i.e., tryptone, consisted of four levels: T0 (control or no tryptone), T1 (1 g/L), T2 (2 g/L), T3 (3 g/L).

The determined tryptone concentration was followed previous study by Silva (2014), which suggests 2 g/L of tryptone for MS enrichment to enhance the growth and development of *Protocorm Like Bodies* (PLBs) in *Cymbidium*. While the concentration of tomato juice used refers Dwiyani et al. (2015), i.e., tomato juice concentration of 100 mL/L for stimulating explant growth.

Tomato juice was prepared in a 1:1 ratio (1000 g of tomatoes mixed with 1000 ml of distilled water) and subsequently blended until it achieved a smooth consistency. The resulting tomato pulp was filtered into an Erlenmeyer flask to obtain a stock solution of tomato juice with a concentration of 100%. This tomato juice stock was then utilized to create treatments of 50 mL/L and 100 mL/L.

The variables observed were germination percentage, germination rate, plant height, number of leaves, number of branches, leaf width, and root length. The experimental design used was a CRD with four replications with a total of 96 experimental units. The data obtained was tested using analysis of variance or ANOVA. Variations between treatment means were analyzed using the Duncan Multiple Range Test (DMRT) with an error rate of 5%.

## Results and Discussion

**Germination Percentage.** Germination percentage is a parameter used to measure the success rate or the percentage of seeds that successfully germinate in this research. The analysis of variance showed that each treatment of tomato juice and tryptone affected the percentage of strawberry germination (Table 1).

The analysis showed that the highest germination percentage occurred in the treatment without tomato juice (0 mL/L), with a result of 0.52%. Applying 50 mL/L and 100 mL/L of tomato juice significantly reduced the germination percentage. The reduction in germination percentage is associated with the inhibitory properties of tomato juice, which contains organic acids known to have a growth-retarding effect (Bidhari et al., 2018). In line with research conducted by Yunita et al., (2023), the utilization of 12 mL/L tomato extract led to a

reduced number of shoots compared to the absence of tomato extract application. Excessive application of tomato fruit extract can lead to an imbalance of nutrients in the growth medium. While tomato fruit extract may contain beneficial nutrients for plant growth, such as vitamins and minerals, an overabundance of certain components may disrupt nutrient uptake and utilization by the plants, thereby hindering their growth (Mastuti et al., 2017). Additionally, its acidity and the presence of caffeic acid and ferulic acid are linked to the suppression of plant growth (Bubna et al., 2011).

The use of 1 g/L tryptone led to a decreased germination percentage, although it did not differ significantly from the application without tryptone. However, increasing tryptone concentrations to 2 and 3 g/L further decreased the germination percentage. This aligns with research findings of Kovács et al. (2012) that high concentrations of amino acids in the growth medium can induce osmotic stress on plants. So that, excessive amino acids may limit the plant's access to essential resources needed for growth.

**Table 1. The effect of various concentrations of tomato juice and tryptone on the germination percentage of strawberry explants**

Treatment	Germination percentage	
<b>Tomato juice</b>		
0 mL/L	0.524	a
50 mL/L	0.333	b
100 mL/L	0.290	b
<b>Tryptone</b>		
0 g/L	0.528	a
1 g/L	0.421	a
2 g/L	0.360	ab
3 g/L	0.222	b

Note: Numbers followed by different letters in the same factor of treatment show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%

**Germination Rate.** The analysis of variance showed that tomato juice affected the percentage of strawberry germination (Table 2).

The highest germination rate was found in the tryptone treatment with a concentration of 2 g/L, without tomato juice. Haghighi et al., (2022) explained that as an amino acid, tryptone plays a role in stimulating plant growth and development. Al-Juthery et al., (2019) also added that Amino acids are important in the synthesis of hormones and secondary metabolites. In addition, amino acids play a role in the

formation of coenzymes and play a role in plant metabolism. Amino acids contribute to increasing the ability to absorb nutrients from the growing medium to increase vegetative growth (Baqir et al., 2019).

The application of tomato juice without tryptone was not able to increase the germination rate in strawberry seeds. However, when tomato juice was combined with tryptone, it resulted in a decrease in the germination rate across all treatment combinations. Tomato juice and amino acids can serve as substrates for microbial growth in the growth medium. This result related with the research of Saepudin et al. (2020), applying organic materials to the media can elevate microbial contamination. Microbial activity may alter the chemical composition of the medium, produce metabolites that affect plant growth, or compete with plants for resources. Changes in microbial communities or metabolite production can influence plant growth negatively.

**Table 2. The effect of various concentrations of tomato juice and tryptone on the germination rate of strawberry explants**

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	0.57 A b	0.61 A b	1.05 A a	0.97 A a
50	0.70 A a	0.33 B bc	0.63 B ab	0.12 C c
100	0.70 A a	0.65 A a	0.37 C b	0.58 B ab

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

**Plant Height.** Enhancing plant height is a physiological process involving cell division and elongation. This process is a form of the influence of plants on the surrounding environment. The results of the analysis of variance showed that there was a significant effect on the treatment of tomato juice and tryptone on strawberry plants. The effect of tomato juice and tryptone concentrations on strawberry plant height is presented in Table 3.

Medium enriched with 50 mL/L tomato juice and without tryptone gave the best results for plant height, namely 6.78 cm, but it is not significantly different from without the application of tomato juice. This is related to the cytokinin content in tomato juice which actively plays a role in cell division and bud formation (Setiari et al., 2016). In addition, tomatoes

contain phosphorus, calcium, iron, tyramine, vitamins A, C and K, which can increase plant growth and development (Semiarti et al., 2010).

**Table 3. The effect of various concentrations of tomato juice and tryptone on the strawberry plantlet height**

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	4.60 B a	1.62 A b	4.76 A a	2.70 A b
50	6.78 A a	0.41 A b	0.41 B b	0.11 B b
100	0.65 C a	0.70 A a	0.78 B a	0.72 B a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

**Number of leaves.** The results of the analysis of variance showed that there was a significant effect on the treatment of tomato juice and tryptone on strawberry plants. The effect of tomato juice and tryptone concentrations on number of strawberry leaves is presented in Table 4.

**Table 4. The effect of various concentrations of tomato juice and tryptone on the number of leaves of strawberry plantlet**

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	3.37 A a	0.37 A b	3.50 A a	1.62 A ab
50	3.62 A a	0.12 A b	0.12 B b	0.00 A b
100	0.12 B a	0.75 A a	0.25 B a	0.75 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 4 showed that the of tomato juice and tryptone concentrations was able to produce the highest number of leaves. However, the number of leaves of plantlets grown on MS 0 + 50 mL/L tomato juice and MS 0 + 2 g/L tryptone media was similar compared to plantlets grown on MS 0 media. Plantlets with the higher average number of leaves were found in MS 0 medium, medium enriched with 2 g/L tryptone, and medium enriched with 50 mL/L tomato juice respectively 3.37; 3.50; and 3.62 leaves. The increase in the number of leaves is expected to enhance plantlet growth due to the higher energy generated through photosynthesis.

The medium enriched with 50 mL/L of tomato juice, but no real difference from controls, resulted the highest number of leaves because tomato juice serves as a source of

vitamins, fats, proteins, and growth regulators, including cytokinin. The cytokinin hormone is involved in leaf development and can influence the process of cell division in meristem tissue. This is in accordance with the research from Mokoginta et al. (2021) that cytokinin application in *in vitro* culture of *Dendrobium* sp. orchids can induce shoots and increase the rate of plant multiplication, where cytokinin play a role in plant cell division activity.

Medium enriched with 2 g/L tryptone increases the number of leaves because tryptone, as a source of amino acids play critical roles in various cellular processes, including cell division, elongation, and differentiation (Takatsuka & Umeda, 2014). During leaf formation, nitrogen is incorporated into proteins that are involved in structural components of cells, enzymes, and regulatory proteins (Shah et al., 2017) necessary for leaf growth and development.

**Number of branches.** Branches are shoot organs that extend laterally and function to help supply carbohydrates to the plantlets. The analysis of variance showed an influence of tomato juice and tryptone on the number of strawberry explant branches (Table 5).

**Table 5. The effect of various concentrations of tomato juice and tryptone on the number of branches of strawberry plantlets**

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	3.37 A a	0.37 A b	3.50 A a	1.62 A ab
50	3.62 A a	0.12 A b	0.12 B b	0.00 A b
100	0.12 B a	0.75 A a	0.25 B a	0.75 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 5 showed that the combination of tomato juice and tryptone concentrations was

able to produce the highest number of branches, with an average number of leaves of 3.625 found in MS 0 medium, medium enriched with 2 g/L tryptone, and medium enriched with 50 mL/L tomato juice respectively 3.37; 3.50; and 3.62 leaves.

Medium enriched with 50 mL/L tomato juice showed that branches reached 3.62, but not significantly different from tryptone 2 g/L. The increase in the number of branches is related to the cytokinin content in tomato juice. Aiman et al. (2022) also said that the cytokinin ZPT contained in tomato juice accelerates the cell division process, producing more branches. This follows the opinion of Wróblewska (2013) that growth regulators, especially cytokinins, can stimulate branching (axillary buds) growth by triggering apical buds' dormancy to produce branches.

Medium enriched with 2 g/L tryptone increased the number of leaves. This is related to amino acids, which can function to improve nutrient availability and uptake, which can increase photosynthetic activity and photosynthate accumulation (Wróblewska, 2013). Amino acids are the building blocks of proteins, including enzymes involved in photosynthesis such as rubisco. Adequate amino acid availability supports the synthesis of these enzymes, optimizing photosynthetic efficiency (Rydzys et al., 2021).

Figure 1. illustrates that applying 50 mL/L of tomato juice without tryptone results in a broader leaf area, whereas applying 2g/L of tryptone without tomato juice leads to a higher number of branches. This suggests that the tomato extract content, particularly the phytohormone cytokinin, may stimulate the development of wider leaves, while the application of tryptone as a source of amino acids, the precursors of proteins, can enhance branching. Proteins play a crucial role in various biological functions, including cell structure and plant metabolism.



**Figure 1. a. Plantlet growth on MS0 medium; b. Plantlet growth on MS0 + 50 mL/L tomato juice c. Plantlet growth on MS 0 + 2 g/L tryptone**

**Leaf width.** The analysis of variance showed an influence of tomato juice and tryptone on the leaf width of strawberry explants (Table 6).

**Table 6. The effect of various concentrations of tomato juice and tryptone on the leaf width (cm) of strawberry plantlets**

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	1.77 B a	0.24 A b	1.78 A a	0.54 A b
50	2.55 A a	0.00 A b	0.05 B b	0.00 A b
100	0.05 C a	0.24 A a	0.00 B a	0.35 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 6 shows the broadest leaf width in the medium treatment enriched with 50 mL/L tomato juice, namely 2.55 mm. Bidhari et al., (2018) reported that the application of tomato extract also increased the length of banana cv Ambon leaves under *in vitro* condition. Malinda et al., (2022) also stated that the auxin content in tomatoes can help the process of organogenesis, somatic embryogenesis, and shoot growth in micropropagation.

Large leaf area increases the rate of plant photosynthesis. Photosynthate will support plant tissue cells' work, thereby accelerating growth and development, for example, leaves, stems, and roots (Hemon et al., 2022). The accumulated photosynthate forms new organs and is proven by the plant height and leaf area being higher than others.

## Conclusion

Applying 50 mL/L tomato juice increased plantlet height, number of leaves, branches, and leaf width but reduced the germination percentage. In addition, applying 2 g/L tryptone increased the germination rate and number of leaves and branches of strawberry plantlet under *in vitro* condition.

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## Metallic light-based bird-repellent prototype to reduce paddy yield loss in Jatinangor, Sumedang Regency

**Abstract.** Munia birds (*Lonchura* spp.) are pests that attack paddy plants during the reproductive phase. The traditional methods of controlling birds are considered less effective because birds can adapt to the tools used. This study aimed to determine the effectiveness of a metallic light-based bird-repellent prototype to reduce paddy yield loss. The experiment was conducted in Cileles Village paddy fields, Jatinangor Subdistrict, Sumedang Regency, West Java Province, Indonesia. The effectivity of the prototype was tested by measuring the effective distance (m<sup>2</sup>), counting the frequency of bird visits (number of visits), bird populations, and the level of paddy damage (%), and paddy yield (kg/64 m<sup>2</sup>). The result of the experiment showed that 3 m was an effectual deterrent factor for the prototype, thus the tool can protect paddy plants with an area of 28.26 m<sup>2</sup>. The frequency of bird arrivals in the treatment was 22.25 times, compared to 61.25 times in the control; the bird population in the treatment was 48.25 birds, while the control was 108.75 birds; the level of damage to paddy panicles in the treatment was 10.11%, compared to 37.79% in the control; and the production of paddy yield with the repellent was higher (33.37 kg/64 m<sup>2</sup>) than the control (23.09 kg/64 m<sup>2</sup>). This innovative prototype was potentially able to deter bird pests in the paddy fields.

**Keywords:** Antipredator · Bird deterrent · Paddy protection · Reflectors

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## Introduction

Paddy (*Oryza sativa* L.) is an essential commodity for Indonesians. Statistics Indonesia (2022) states that the population in Indonesia currently reaches 278 million people and most of them use paddy as a staple food. Indonesia's population will increase over time, resulting in a greater demand for paddy (Nuriliana, 2021). However, efforts to increase paddy production in Indonesia have been hampered by several factors, including Plant-Disrupting Organisms (PESTs). According to Sumayanti (2021), birds are one of the pests that can cause direct damage to paddy plants during the generative phase.

Munia birds (*Lonchura* spp.) are grain-eating animals that can cause damage to paddy plants. According to Bari et al. (2021), munia birds that attack paddy plants consist of Javan Munia (*Lonchura leucogastroides*), Spotted Munia (*L. punctulata*), and White-headed Munia (*L. maja*) with an average body size of around 10-11 cm (Joshi, 2019). Munia birds show daily feeding activities by 16.9-44.69% and moving and resting activities by 35.9-65.2% (Dwijayanti et al., 2021). As a result of their feeding activities, munia birds can reduce paddy production by 50% and cause losses of Rp. 2,500,325/ha in Bogor City (Ziyadah, 2011; Ardjansyah et al., 2017).

Munia bird control in paddy crops can be applied using various techniques. In general, farmers use scarecrows or cans filled with gravel at an indeterminate distance and guarded for a full day (Ejiogu & Okoli, 2012). These methods are decreasing in effectiveness due to frequent use, and birds adapt to scarecrows or cans filled with gravel. Birds have antipredator traits that will be active when disturbed by shadows or sounds they are not used to hearing (Beauchamp, 2015).

Reflectors are one of the tools that can induce antipredator traits in birds. The reflector will reflect light and affect bird vision (Emerson et al., 2022). The use of reflectors will be more effective if combined with predator shapes and the addition of sound (Bishop et al., 2003). Currently, no studies evaluate the effectiveness of predator-shaped reflectors combined with additional sound in paddy fields to deter birds. Therefore, this study aims to evaluate the effectiveness of a metallic light-based bird-repellent prototype to reduce paddy yield loss.

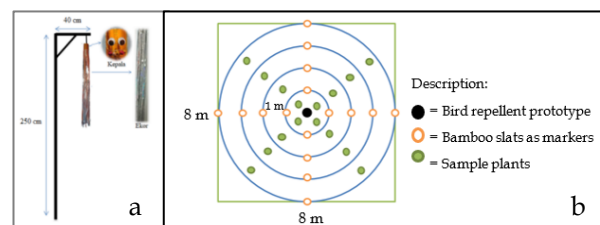
## Materials and Methods

**Research site.** The experiment was conducted from February to May 2020 in a farmer-owned paddy field in Cileles Village, Jatinangor District, Sumedang Regency, at 6°54'35.4 "S, 107°46'31.5 "E with an altitude of 750 m above sea level. The experiment was conducted during the dry season with an average wind speed of 10 km/h from the west direction. The area used for testing was a paddy field with paddy plants that had entered the generative phase (age  $\geq 70$  days) and had a high level of bird infestation. Each research plot measured 64 m<sup>2</sup> with a distance of 2 m between the plots.

**Bird repellent preparation.** The research uses an experimental method of comparing two means (Independent Samples t-test) consisting of two treatments, with the installation of repellent devices and without repellent devices (control). Each treatment is repeated seven times.

The repellent preparation was initiated by throwing away the top and bottom lids of milk cans. The outside of the can is covered with gold-colored foil paper 25 x 10 cm. A 2 m x 1 m metallic curtain was divided into two parts (1 m x 1 m) and attached to the inside of the can. The device is then attached with rattles and eye-shaped accessories to the top of the can. Five rattles ( $\varnothing$ : 1.8 cm) first be inserted into the u-shape wire with the hooked two ends to the top of the can to form the lip of the device.

The bird repellent was placed in the center of the paddy field by hanging it with a 2.5 m high pole (Figure 1a). A stake was placed per meter from the repellent device to mark the distance or radius from the center (Figure 1b). Sampling was carried out using the diagonal line method to represent each area of each radius.



**Figure 1.** The shape of the bird repellent tool and its placement pattern. (a), the pattern of repellent placement, and paddy sampling (b).

**Bird species identification.** Bird species observation was conducted by directly observing birds attacking paddy plants and documented using an observation camera (yi outdoor camera 1080p). Bird identification uses bird guidebooks (Field Guide Series of Birds in Sumatra, Java, Bali, and Kalimantan by MacKinnon et al. 2010).

**Effective distance of bird-repellent devices.** Observations were made using an observation camera and direct observation. The observation camera was kept in the corner of the research field to show all areas of the research field from 6:00 am to 06:00 pm. Observations of the effective distance of the repellent prototype were made by determining the distance at which birds visited paddy plants in the research plot and by counting the number of birds at each repellent radius. Observations of the effective distance of the repellent prototype were made by determining the distance at which birds visited paddy plants in the research plot and by counting the number of birds at each repellent radius.

**Visit frequency and bird population.** The frequency of bird visits was calculated by summing the number of bird groups visiting the research plots. Meanwhile, the bird population was calculated by summing up the total number of birds that came to the research plot area through a camera that had been installed every day for 12-hour observations.

**Percentage of paddy damage.** According to Ardjansyah et al. (2017), the level of damage to paddy caused by birds can be done by direct observers to the clumps marked as samples and then counting the number of intact and non-intact panicles. This calculation uses the following formula:

$$\% \text{ panicle damage} = \frac{y}{x} \times 100\%$$

Note:

y= Number of panicles attacked

x= Total number of panicles

**Paddy yield.** Paddy yield data was obtained at the end of the experiment. Paddy yield loss is determined using the following formula: Potential yield of the IR36 variety (kg/64 m<sup>2</sup> - production yield in the field (kg/64 m<sup>2</sup>). Loss of harvest yield and harvest yield per plot are then compared between the treatment and control.

**Data analysis.** Data were analyzed using the Independent Sample T-Test method, an average difference test with two independent data that were repeated seven times. Subsequently, the data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software version 21.

## Results and Discussion

**Bird Species Identification.** The type of bird that attacks paddy plants is generally the munia bird group (*Lonchura* spp.). Bari et al. (2021) report that Javan Munia (*L. leucogastroides*), Spotted Munia (*L. punctulata*), and White-headed Munia attack paddy plants (*L. maja*). The paddy planted in the research field is the IR36 variety. Based on observations, only Javan Munia was found attacking paddy plants in the research paddy fields (Table 1).

During the observation, birds flying in the study area were characterized by dark brown heads and tails, white bellies, and chests. These characteristics refer to the Javan Munia (*L. leucogastroides*) described in the Field Guide to Birds of Sumatra, Java, Bali, and Kalimantan by MacKinnon et al. (2010). Javan Munia can adapt to various habitats and has a rapid reproductive rate, allowing it to dominate the study area (MacKinnon et al., 2010). Other types of munia birds were not found. In addition, there were sparrows found in the yard area of residents' houses or perched on electric cables on the edge of paddy fields.

**Table 1. Results of Bird Species Identification in the Study Area**

Bird Species	Characteristics according to MacKinnon et al. (2010)	Observation Results
Javan Munia ( <i>L. leucogastroides</i> )	The feathers are dark brown from the head to the tail, and the belly is white.	Found
Spotted Munia ( <i>L. punctulata</i> )	The feathers are brown on the upper part of the body and white on the lower part, with spotted patterns on the chest and sides.	Not found
White-headed Munia ( <i>L. maja</i> )	The feathers are dark brown from the neck to the tail, while the head to the neck has white feathers.	Nor found

The Javan Munia feeds primarily on grains such as paddy or grass seeds and uses large trees as nesting sites. Javan Munia is common in Java, Sumatra, NTT, NTB, and Bali (Coates & Bishop, 2000; Sulistyadi, 2010). This bird's feeding habits form groups in large and small numbers (MacKinnon, 1990). According to Hidayatullah (2015), the nesting characteristics of the Javan Munia are in trees with a height between 3.5-8 m, a nesting height of 1.5-4.5 m, and a canopy area of 2.5-109.7 m<sup>2</sup>. Javan Munia utilizes soursop trees (*Polyalthia muricata*), mango trees (*Mangifera indica*), and water guava trees as nesting sites (*Eugenia aquea*).

**Effective Spacing of Bird Repellent Prototypes.** The bird-repellent prototype was placed at the center of the research field, and every one-meter distance was marked using a stake. Based on the observations, there were differences in the number of individual birds that attacked at each radius (Table 2).

**Table 2. Javan Munia Population in the Treatment Field**

Observation Time	Bird Population at Radius to... (birds)				Total (birds)
	0-1	1-2	2-3	3-4	
	m	m	m	m	
11 WAP	-	-	-	24	24
12 WAP	1	2	4	38	45
13 WAP	2	3	9	35	49
14 WAP	4	7	20	44	75

\*WAP: Week After Planting

Table 2 shows that on the 11th WAP, the visitation of munia birds was minimal due to abundant food sources in the research area, leading the birds to evenly distribute their visits to other fields not designated for research. Additionally, the number of birds at each radius within the repellent treatment area varies. The number of individual birds at a distance of 3-4 m from the repellent had the highest value than at closer distances. This phenomenon happened because, at a radius of 1-3 m, flashes of light from the metallic curtain and the sound of rattles and strands of the metallic curtain can protect the paddy from bird attacks. These sounds and flashes activate the adrenal cortex, causing bird fear (Davis et al., 2008).

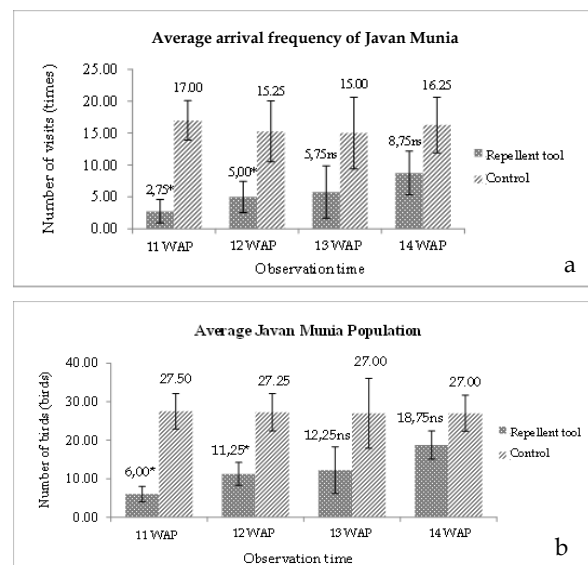
In addition, the presence of trees that served as bird perches around the research area caused birds to attack paddy on the side of the field closest to them (3-4 m from the repellent device). These results align with the research of Muslihun

(2010), which states that the highest intensity of bird attacks occurred at the edge of the plantation at 62.23%, compared to the middle part at 29.54% and the middle part at 24.07%.

#### Arrival Frequency and Bird Population.

Bird arrival frequency was lower in the treated plots compared to the untreated plots when using the bird-repellent prototype. Around the observation area, there are no various other types of plants that could influence bird behavior such as cassava plantation. It was observed that the birds did not cause any damage to the cassava crops. This could be associated with Milolo et al. (2023), that the birds able to not to eat crops due to the absence of food preference. Due to the paddy cultivation in the observation area, which is the primary preference of the Javan Munia, it will refrain from consuming other crops.

The total average arrivals frequency in the treatment plot was 22.25 times, while in the control plot was 61.25 times (Figure 2a). The average Javan Munia bird population in the treatment plot was 48.25 birds, while in the control plot reached 108.75 birds during the period (Figure 2b).



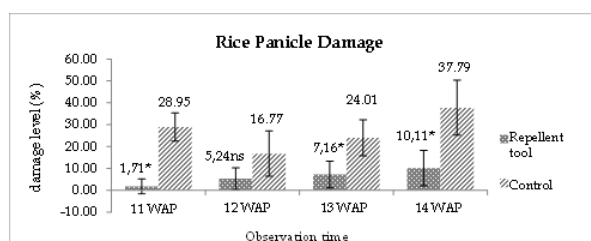
**Figure 2. Arrival frequency and bird populations. Frequency of arrival of Javan Munia birds (a) and the average number of Javan Munia individuals (b) in paddy fields with treatment (prototype repellent) and without treatment (control).**

Notes: (\*) Significantly different. (ns) Not significantly different.

The lower arrival frequency and bird populations in the treatment field is thought to be because the repellent can trigger antipredator traits in birds. This trait can emerge when birds

see artificial predators or sound generated from an object in the center of a field (Storms et al., 2022; Kasmawan et al., 2018). The prototype repellent used is thought to look like a predator, creating light reflections from the metallic paper like a reflector, affecting the birds' vision. This is consistent with the findings of Khan's (2014) study, which demonstrated that the number of crows visiting the wheat nursery decreased to zero after being treated with reflector tape. In contrast, the number of crows, parakeets, and mynah birds decreased in corn entering the milk maturity phase after applying reflector tape (Khan, 2014).

**Paddy Panicle Damages.** Based on the study's results, damage to paddy panicles in control plots fluctuated over time (Figure 3). Damage to paddy panicles at 11 WAP reached 28.95%, which was thought to be due to the paddy grains entering the milk ripening phase. This phase is the preferred phase of Munia birds, where the juvenile grain of paddy is chopped using their beaks, then the birds will suck the thick white liquid and then remove the empty seed coat (Bari et al., 2021). Panicles damage at 12 WAP decreased because due to the transition from the milk stage to the ripe stage. This makes it difficult for birds to eat the paddy.



**Figure 3. The average level of panicles damage by the Javan Munia bird.**

**Notes:** (\*) Significantly different. (ns) Not significantly different.

The use of the repellent prototype showed lower levels of panicle damage compared to the control. This is thought to be the effect of the combination of flashes of light, the sound of curtains blowing in the wind, and the sound of rattles that drove birds away from the study field. According to Bishop et al. (2003), adding sound in reflector-based repellents will be more effective in controlling birds. The panicle damage caused by birds in the prototype repellent treatment increased over time. This is thought to

be due to the adaptation process of Munia birds to the repellent used. Javan Munia can adapt to various kinds of habitats and reproduce at a rapid rate, resulting in a gradual increase in paddy plant damage (MacKinnon et al., 2010).

**Average Yield of Paddy.** Using the repellent prototype affected the level of damage to paddy panicles, which correlated with the average of yield production. Paddy fields treated with the repellent prototype showed a significantly higher average yield than the control (Table 3). This phenomenon happened because the paddy plants in the control field were eaten by Javan Munia birds.

**Table 3. Effect of using the repellent prototype on the average yield of paddy plant production**

Treatment	Potential yield (t/ha)	Potential yield (kg/64 m <sup>2</sup> )	Loss of yield (kg/64 m <sup>2</sup> )	Paddy Yield (kg/64 m <sup>2</sup> )
Prototype of repellent tool	5.8	37.12	3.75*	33.37*
Control	5.8	37.12	14.03	23.09

Description: (\*) Significantly different.

The yield potential of the IR36 variety is 5.8 t/ha (Suprihatno et al., 2009) and converted to 37.12 kg/64 m<sup>2</sup>. On 64 m<sup>2</sup> of research land, yield loss due to bird infestation in the prototype repellent treatment plots was lower than the control, which correlated with the number of yield productions. This is in line with research by Hardiansyah et al. (2023), which showed that the average yield of paddy can increase by up to 89% when using repellent compared to without repellent.

## Conclusion

An innovative prototype repellent made from metallic curtains and rattles effectively controls birds that attack paddy plants. It could suppress bird visits up to 3 m. In addition, the repellent prototype reduced the frequency of visits and bird population in the study area. As a result, the panicle damage caused by the prototype repellent treatment plots was significantly lower than that of the control. In addition, the use of the repellent was able to produce higher average yields than the control in paddy plants.

## Acknowledgments

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## Growth and yield response of Tanjung II and Unpad CB2 red chili varieties grown in the medium land

**Abstract.** Red chili is an important commodity that is daily needed by the community. Non-central chili areas often experience deficits due to the high consumption volume and low productivity of chili peppers. To increase the productivity of chili in non-central areas, expand it in the medium land. This study aims to determine the productivity of two introduced chili varieties in the medium land whose adaptation areas came from lowland and highland areas. This study was analyzed using an independent sample T-test at a significant level of 5%. The results of the data analysis showed that the Tanjung II variety significantly influenced the parameters of stem diameter and weight of the fruit. Meanwhile, the Unpad CB2 variety significantly influences the parameters of plant height, number of leaves, number of flowers, number of fruits, and fruit set. However, both varieties have no noticeable effect on the weight parameters of the cropping fruit and potential yield in hectares. The Tanjung II variety experienced a decrease in yield by 54.58%, while the Unpad CB2 variety was 53.56%.

**Keywords:** Expand · Medium land · Productivity · Red Chili · Variety

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## Introduction

Red chili (*Capsicum annuum* L.) is one of the most strategic horticultural commodities used as agricultural products. Red chili has an important role in meeting the needs of the community. Fluctuating chili prices have an impact on the formation of inflation. One of the reasons for the fluctuation in chili prices is the season and the availability of products in the market (Romeida et al., 2020).

In Indonesia, red chili domestic production in 2019 reached 1.21 million tons (Statistics Indonesia, 2020), with consumption needs of 368.160 tons in 2014, while the development of export volume during the period 2006-2014 averaged 936.090 tons (Yanuarti & Afsari, 2016). These data indicate that chili production is still in deficit or shortage, causing trade between regions (Statistics Indonesia, 2020). In areas of non-production centers, of course, they will buy from areas of production centers so that a distribution chain is formed.

Efforts to increase chili productivity in non-central areas can be made using intensification, namely increasing production by genetic engineering and cultivation patterns on the same land area. In addition, it can also carry out extensification by expanding the chili cultivation area. Highland areas are no longer a consideration because they are more suitable for conservation land than cultivated land (Juarsah, 2017), so agricultural cultivation practices need to optimize land in low to medium-land areas.

Chili is a horticultural product that is perishable and consumed fresh. Due to high temperatures, it is very risky if chili is planted in lowland areas. High temperatures cause plants to wilt quickly and lose water in the field and during the post-harvest process. Therefore, the medium land can be a solution for expanding the red chili plantation area because of the chili's adaptive characteristic to the plantation area on medium land that is not frosty such highland and not very warm such lowland areas. Chili can grow well at an altitude of 0-1400 meters above sea level, although not wide varieties can grow well in these areas.

The Tanjung II variety belongs to the segregated red chili, with an adaptation area in the lowlands, with an earlier age, almost simultaneous maturity time, resistance to trips and aphids, low seed prices, easy to sell in the local market, and

good fruit color. Interesting if used as a paste (Basuki et al., 2014). Meanwhile, the Unpad CB2 chili variety is a red chili that was previously crossed with cayenne pepper, so the spiciness level is higher than red chili in general. The Unpad CB2 variety has an adaptation area in the highlands, with its fruit shape resembling a curly chili and a high yield of 23.6 tons/ha. This study aims to determine the productivity of two introduced chili varieties in the medium land whose adaptation areas came from lowland and highland areas.

## Materials and Methods

This research was conducted in a plastic house in Sindanggalih Village, Cimagung District, Sumedang Regency, West Java, from March to August 2021. The location of this research is at an altitude of 746 meters above sea level with Latosol and Alluvial soil types with a temperature range of 26 – 30 °C (Guswita et al., 2020). According to Istiawan & Kastono (2019), in general the altitude of the place is divided into 3 regions, namely the lowland (<400 m above sea level/masl), the medium land (400-700 masl) and the highlands (>700 masl). Almost the same as the height classification contained in Permentan No. 47/Permentan/OT.140/10/2006 that mountainous land based on elevation is distinguished from medium land (350-700 masl) and highlands (>700 m asl). The height of the place is closely related to the type of plant that is suitable for maintaining environmental sustainability.

The tools used were scissors, a ruler, a marker, a meter, a sprayer, and a digital caliper. The materials used were warm water, soil, husk charcoal, chicken manure, Tanjung II and Unpad CB2 chili seeds, seed tray, polybag (40 cm x 50 cm), stakes, UV plastic, labels, plastic rope, Gandasil D fertilizer and B, NPK Mutiara, urea, KCl fertilizers. Pesticides in form of Curacron, Sidamethrin, Demolish, and Antracol were also used.

Seedling is done by sowing chili seeds soaked in warm water (50 °C) for 1 hour. The planting media used during seeding and planting were 40% soil, 30% chicken manure, and 30% husk charcoal. Transfer of seedlings to polybags is carried out 21 - 28 days after sowing or when the seedlings have 4 - 5 true leaves. The maintenance includes fertilizing once every ten days, watering once a day, replanting if the

plants are withered, installing stakes to support the chili plants so they do not fall over easily approximately two weeks after planting (WAP), loosening the soil, pinching in two WAP, and controlling plant-disturbing organisms such as plants' weed, pests, and diseases. Red chilies are harvested by computational methods, namely counting from the time of planting/flowering and visually when the chili is red from the base to the tip of the fruit. Computationally harvested chilies calculated from 50 - 60 days after flowering.

The parameters observed consisted of growth parameters namely plant height (cm), number of leaves (strands), and stem diameter (mm) measured at 3, 4, 5, and 6 WAP. The yield parameters consist of the number of flowers, number of fruits, fruit set (%), fruit weight (g), fruit weight of one plant (g), and potential yield per hectare (tons/ha). Each variety consists of 15 replicates, each replication containing three samples, so the total number of plants is 90 samples. Data were analyzed by independent sample T-test using Statistical Program for Social Science (SPSS) version 20 software at a 5% level.

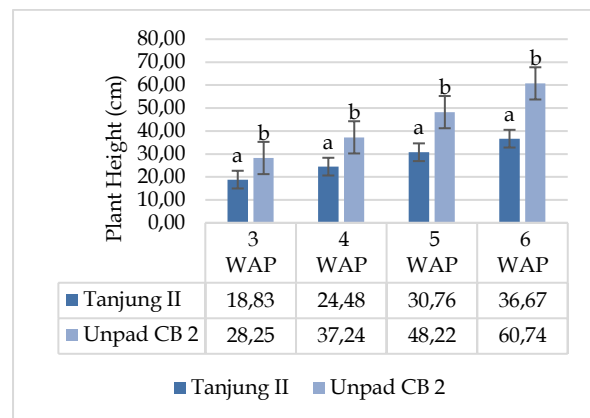
## Results and Discussion

**Plant Height (cm).** Figure 1 shows Unpad CB chili varieties have a higher average plant height at 3-6 WAP than Tanjung II. The increase in plant height of the Tanjung II variety was 30.04% at 4 WAP, 25.61% at 5 WAP, and 19.22% at 6 WAP. The Tanjung II variety experienced an increase in plant height by at least 6 WAP because the plant had entered the generative phase. The vegetative growth began to decline. In addition, plants are also attacked by aphids which attack the growing point in the form of shoots, causing stunted plant growth.

Meanwhile, Unpad CB2 red chili, according to the description, was able to grow to a height of 73.4 - 87.5 cm. However, at 6 WAP, the height of the chili plant was only 60.74 cm. Unpad CB2 chili can still experience high growth due to the increase experienced when 6 WAP was still quite large, namely 25.97%. Chili can still grow taller at 7 or 8 WAP until it reaches its optimal height.

Figure 1 shows that the difference in varieties significantly affected the height of chili

plants from 3 WAP to 6 WAP. The Unpad CB2 variety produced higher plant height than the Tanjung II variety.

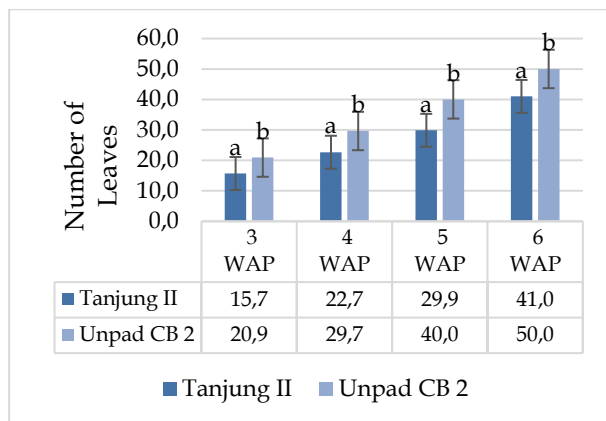


Note: The average value followed by the same letter in each row shows that it is not significantly different based on the unpaired T-test.

**Figure 1. Average Plant Height of Tanjung II and Unpad CB2 Varieties Grown in Medium land**

Different genetic characteristics and phenotypes caused the difference in plant height. As stated by Kusuma et al. (2009) in Naibaho et al. (2021), different parental crosses influenced different plant heights in each variety. Each variety's adaptability is different in terms of environmental factors and growing media (Dermawan et al., 2019). The Tanjung II chili variety adapts well to lowland environments, and the Unpad CB2 variety adapts to the highlands. The experiments carried out on both chili peppers were in the medium highland. So environmental changes affect plant height growth (Fauzi & Subositi, 2019).

**Number of Leaves.** Figure 2 shows that Unpad CB chili varieties have an average number of leaves at 3-6 WAP than Tanjung II. The number of chili leaves of the Tanjung II variety was 44.41% at 4 WAP, 31.73% at 5 WAP, and 37.25% at 6 WAP. The Tanjung II variety experienced an increase in the number at least 5 and 6 WAP. Meanwhile, Unpad CB2 red chilies increased the number of leaves at 4, 5, and 6 WAP, which were 41.72%, 34.98%, and 24.92%. The percentage increase in the number of leaves in both varieties tends to decrease as the plant approaches the generative phase. The vegetative growth begins to decline. In addition, the environment in which it grows can also affect plant leaf growth (Fauzi & Subositi, 2019).



Note: The average value followed by the same letter in each row shows that it is not significantly different based on the unpaired T-test.

**Figure 2. Average Number of Leaves of Tanjung II and Unpad CB2 Varieties Grown in Medium Highland**

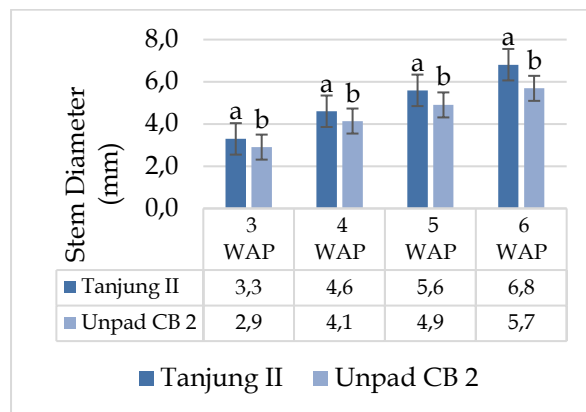
Figure 2 shows that the difference in varieties significantly affected the number of chili leaves from 3 WAP to 6 WAP. The Unpad CB2 variety produced more leaves than the Tanjung II variety.

The tolerance of Tanjung II chili varieties to aphids did not apply if the plants were planted in the medium highland. Aphids can grow optimally in the dry season with a temperature range of 25-30 °C (Utama et al., 2017). Pest attacks in the form of aphids and trips caused leaf growth to become stunted, and the number of leaves formed was small. Pest trips and aphids cause plant leaves to curl, shrivel, and cause leaf growth to be stunted (Meilin, 2014). The difference from the trip's pest attack is that the plant leaves will curl up, while the aphids will curve down. Both can appear when environmental conditions enter the dry season and actively attack at the beginning of plant growth until they enter the generative phase (Meilin, 2014).

**Stem Diameter (mm).** In Figure 3, it shows that the chili varieties of Tanjung II have a larger average stem diameter at 3-6 WAP than those of the Unpad CB2 variety. The increase in stem diameter of the Tanjung II variety was 39.70% at 4 WAP, 21.53% at 5 WAP, and 21.71% at 6 WAP. The Tanjung II variety experienced an increase in the number of at least at 5 and 6 WAP. Meanwhile, Unpad CB2 red chilies increased stem diameter at 4, 5, and 6 WAP, which were 42.48%, 18.45%, and 15.99%.

Figure 3 shows that the difference in varieties significantly affected stem diameter from 3 WAP to 6 WAP. The Tanjung II variety

significantly affected stem diameter compared to the Unpad CB2 variety.



Note: The average value followed by the same letter in each row shows no significant difference based on the unpaired T test

**Figure 3. Average Stem Diameter of Tanjung II and Unpad CB2 Varieties Grown in Medium Land**

The percentage increase in stem diameter in both varieties tended to decrease as the plant approached the generative phase. The vegetative growth began to decline. In the vegetative phase, assimilate is stored in the plant stem. Plant diameter should be directly proportional to plant height, and a larger diameter tends to be able to store greater assimilate and produce higher production (Flowrenzhy & Harijati, 2017).

**Yield Parameters.** The results of the research on the effect of different varieties on yield parameters in the form of a number of flowers, number of fruits, fruit set, fruit weight, planting weight, and potential yield per hectare are presented in the table. Table 1 shows the results of the analysis using the T-test.

**Tabel 1. Plant height data of Tanjung II and Unpad CB2 varieties grown in medium land**

Parameter	Average	
	Tanjung II	Unpad CB2
Number of Flowers	33.53 a	50.47 b
Number of Fruits	26.93 a	41.60 b
Fruit Set (%)	76.10 a	82.38 b
Fruit Weight (g)	4.90 b	4.18 a
Weight of Harvested Fruit (g)	136.13 a	169.56 a
Yield Potential (tonnes/ha)	5.45 a	6.79 a

Note: The average value followed by the same letter in each row shows no significant difference based on the unpaired T-test



**Number of Flowers.** The results showed that the differences in chili varieties significantly affected the number of flowers. The Unpad CB2 variety produced more flowers than the Tanjung II variety.

Table 4 shows the number of flowers that appear on the Tanjung II and Unpad CB2 varieties. The Tanjung II chili varieties produced fewer flowers, with an average of 33.53 flowers. Compared to the Unpad CB2 variety, with 50.47 flowers. The flowering time of the two chilies also gives rise to flowers at different times. Flowering on the Tanjung II variety began to appear at the age of 29 days after planting (DAP). Meanwhile, flowering on the Unpad CB2 variety appeared at 34 DAP, according to the description, which was between 34 - 45 DAP. Flowering time is necessary to know because it is a component that determines fruiting time and harvest time (Flowrenzhy & Harijati, 2017).

Flowering time and the number of flowers planted are related to the process of plant adaptation to climate change and the ability of plants to survive in a different environment than usual (Flowrenzhy & Harijati, 2017). Genetic factors affect the process of pollination and fertilization as well as the ability to grow the formed embryo (Mochtar *et al.*, 2018). In addition, environmental factors such as temperature and humidity affect the transpiration that occurs in plants to maintain the flowers that have emerged (Suparwoto *et al.* 2021). The experimental results in the field showed that the number of flowers was small due to the attack of aphids that attacked the growing point. The shoots of plants attacked by these pests become unproductive and difficult to produce flowers.

**Number of Fruits.** Table 4 shows that the differences in chili varieties significantly affect the number of fruits. The Unpad CB2 variety produced more fruit than the Tanjung II variety.

The number of fruits planted is the number of fruits that appear and survive until the harvesting process is carried out. The number of fruits that appear is usually directly proportional to the number of flowers that bloom, by what happened to the chili varieties of Tanjung II and Unpad CB2. The Tanjung II variety produced fewer harvestable chilies, which was 26.93 compared to 41.60 Unpad CB2. Compared with the description, the Unpad CB variety has not yet reached the minimum number of fruits planted, which is around 47 fruits; however, the

number of fruits at the time of the experiment is close to the description.

The two varieties have different harvest times. Tanjung II chili was harvested when it entered the age of 80 DAP or 51 DAP; however, this was not by the description, which stated that the Tanjung II variety could be harvested at 58 DAP. Harvest time occurs slower for 22 days. The delay in harvest time is due to agronomic differences, which are the adaptation area of the Tanjung II variety. The chili can grow well in the lowlands with higher temperatures. High temperatures cause faster metabolic processes so that crop production increases and harvest time will be faster (Fauzi & Subositi, 2019).

Meanwhile, in the Unpad CB2 variety, chilies can be harvested from the age of 97 DAP or 63 DAS. The description states that the harvest time ranges from 85 - 90 DAP or 51 - 56 DAS. The harvesting time of Unpad CB chili is also seven days slower if it refers to the time of flowering anthesis.

**Fruit Set (%).** Table 4 shows that the differences in chili varieties significantly affect the fruit set. The Unpad CB2 variety produced a more extensive fruit set than the Tanjung II variety.

The fruit set is the ratio of the number of flowers to fruit. The higher the value of the fruit set, the better the plant is at retaining flowers until they become market-worthy fruit. According to Lawalatta *et al.* (2017), the fruit set on a good chili plant is 80%. Tanjung II chili varieties produced a fruit set of 76.10% and 82.38% for Unpad CB2 varieties. The percentage of fruit set formation in both varieties was optimal because it was close to 80%; even in the Unpad CB variety, it was more than 80%.

**Fruit Weight (g).** Fruit weight is the weight of chili that is harvested fresh in less than one day. The weight of chilies planted is the accumulation of the chilies harvested from the first to the last harvest. Based on table 4, it shows that differences in chili varieties have a significant effect on fruit weight. Tanjung II variety produces greater fruit weight compared to Unpad CB2 variety.

Fruit weight is the average weight produced in one chili. In chili, the Tanjung II variety produces a fruit weight of 4.90 grams, while in the Unpad CB2 variety it is 4.18 grams. Referring to the description for the Unpad CB variety, it can produce a fruit weight of 6.6 - 14.8 grams. This shows a decrease of 36.67% from the

supposed weight. The weight loss can be caused by genetic, environmental, growing media, and fertilization factors that affect metabolic processes in plants.

**Weight of Harvested Fruit (g).** Weight of harvested fruit is an important parameter that determines the amount of fruit produced and is suitable for harvesting in one plant. Based on the results of the study, it was shown that the differences in chili varieties did not significantly affect the weight of the crop.

Suparwoto et al. (2021) stated that the fruit produced by a plant would adapt to the genetic potential of each variety. The greater the weight of the fruit, the higher the potential yield. The Tanjung II variety yielded 136.13 grams of planting weight, while the Unpad CB2 variety yielded 169.56 grams. The weight has decreased from what is stated in the description that Unpad CB2 can produce 430.6 – 702.0 g/plant. The decrease that occurred was 60.62%. Meanwhile, the Tanjung II variety according to the description can reach an average weight of 600 g/plant, resulting in a decrease of 77.31%. The weight of the fruit planted can be influenced by two factors, namely the weight of the fruit and the number of fruits planted; if both of these factors have high yields, the weight of the fruit of the plant will also be high.

**Yield Potential (tons/ha).** Yield potential is the ability of plants to produce their main target organs optimally. Yield potential is the primary goal of a cultivation process to find the number of suitable products for harvesting. Based on the results of the study, it was shown that the differences in chili varieties did not significantly affect the yield potential.

Each chili variety has a different yield potential; the factors that affect the yield potential are the weight of the fruit planted (Suparwoto et al., 2021) and the number of plants in the unit area. The higher the weight of the plant, the greater the potential yield. According to the description, the Tanjung II variety can produce 12 tons/ha chilies, while Unpad CB2 is 14.6 – 23.6 tons/ha. The experiments resulted in yield potential for each variety of 5.45 tons/ha and 6.79 tons/ha. The yield decreased by 54.58% and 53.56%, respectively. A decrease in productivity due to an unsuitable location was also experienced by farmers in the Ciamis area who grow Tanjung II chili varieties in the medium highland (Basuki et al., 2014)

According to Suparwoto et al. (2021), differences in the growth and yield potential of each variety are influenced by the genotypic response to the environment in which it grows. The decrease in yield potential of the two varieties is because they are not adaptive varieties in the medium highland. Planting chilies outside their adaptation areas can provide information about plant tolerance to climate change and productivity (Flowrenzhy & Harijati, 2017). According to Gonzalez-Dugo et al. (2007) in Flowrenzhy and Harijati (2017), the modest decrease in productivity due to changes in metabolism is 20%.

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## Conclusion

The conclusions of this study include the following:

1. The Tanjung II variety that is grown in medium highland shows a higher growth and yield performance than Unpad CB2, especially in the form of stem diameter and fruit weight.
2. The Unpad CB2 variety significantly affects growth and yield parameters, especially in the form of plant height, number of leaves, number of flowers, number of fruit, and fruit set.
3. There were no significant differences between the two varieties in yield parameters such as plant weight and potential yield per hectare. The Tanjung II variety experienced a decrease in the yield of 54.58%, while the Unpad CB2 variety was 53.56%.

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## A comparison of synthetic fungicide and *Trichoderma* spp. applications against clubroot disease on cabbage

**Abstract.** Clubroot is one of the important diseases affecting members of the Cruciferae family. This disease is caused by soil-borne pathogen, called *Plasmodiophora brassicae*. The pathogen produces motile spores called zoospores. This pathogen results in a drop of cruciferous plant productivity. The objectives of this research were to identify specific *Trichoderma* species in three districts in Sumatra Utara and to evaluate the potency of *T. harzianum* (both local species from Berastagi and species developed by Indonesian Vegetable Research Institute (IVEGRI)) to control the disease and then comparing them with the application of synthetic fungicide. The research was conducted in a greenhouse of Research Installation and Application of Agricultural Technology (IP2TP), Berastagi, North Sumatera from April–September 2018, using Completely Randomized Design (CRD) with eight treatments: C1 (control-without *Trichoderma*), C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag), C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag), C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag), C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag), C6 (local *T. harzianum* obtained from corn substrate 2 g/polybag), C7 (local *T. harzianum* obtained from corn substrate 4 g/polybag) and C8 (synthetic fungicide Nebijin). Each treatment contained 10 polybags of plants. These treatments were replicated four times. The results exhibited there were 3 *Trichoderma* species found in Berastagi: *T. harzianum*, *T. viride* and *T. koningii*. Also, 4 g of local *T. harzianum* (corn substrate) has better performance (0% disease incidence and 0% disease severity) compared to other treatments.

**Keywords:** antagonist, biological control, disease severity, pathogen

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## Introduction

Cabbage (*Brassica oleraceae* var. *capitata* L.) is one of exported commodities which has a good prospect to be widely cultivated in Indonesia as the demand of this leafy crop is increasing. Statistics Indonesia (2017) informed that cabbage production in Indonesia fluctuated in 2012–2016. In 2012, the production was 22.56 tons ha<sup>-1</sup>, increased to 22.69 tons ha<sup>-1</sup> in 2013 and 22.75 tons ha<sup>-1</sup> in 2014. However, the production was decreased to 22.33 tons ha<sup>-1</sup> in 2015 and 21.94 tons ha<sup>-1</sup> in 2016, with the decrease percentage reaching up to 5.80%. One obstacle that led to this decline is the presence of clubroot disease caused by the *Plasmodiophora brassicae* Wor. Clubroot is an important soil-borne disease of cruciferous crops throughout the world (Gossen et al., 2013; Päsold et al., 2013). It has been reported to cause high yield loss between 50–100%. Infection 10–20% causing loss 25% (Rennie et al., 2011). In Indonesia, estimation of crop failure in cabbage reached by 88.60% and 5.42%–64.81% to choy sum (Darmiati & Sudarma, 2017).

Infected plants show several symptoms where the main symptom is swollen and distorted roots (Gahatraj et al., 2019). This hypertrophy on roots causes malfunction of the xylem and causes plants to have difficulty absorbing and transporting water and nutrients properly. As a result, plants are stunted, wilt easily, and may have yellowing leaves, leading to premature death (Deora et al., 2013). Soils contaminated by this fungus have always been an obstacle for cabbage plantations due to their high resistance to environmental changes in the soil.

Farmers have been implementing several efforts to control this disease by applying resistant plant varieties, crop rotation, soil solarization, adjusting soil pH, planting trap crops, and the use of synthetic fungicides (Hwang et al., 2012). In Karo Regency, North Sumatra, 90% of cabbage farmers have been applying synthetic fungicides to control this pathogen. However, these chemicals did not significantly control the disease due to the resting spore of *P. brassicae* having a great ability to survive without a host in soil. The intensive use of chemicals exhibited undesirable effects such as water, air, soil pollution, and health problems to humans and animals.

Hasyim et al. (2015) stated that using natural enemies or antagonistic organisms can reduce the

negative impacts caused by the continuous use of chemicals. Several antagonistic fungi, such as *T. harzianum*, *Aspergillus niger*, and *Gliocladium*; antagonistic bacteria *Bacillus subtilis*, *B. polymyxa*, *B. thuringiensis*, *B. pantothenicus*, *Burkholderia cepacia*, and *Pseudomonas fluorescens* (Soenartiningih et al., 2014; Sun et al., 2017; Antastia et al., 2019) have been assessed for their biocontrol activities against harmful pathogens. *Trichoderma* spp. cause growth inhibition by microparasitism, antibiosis and competition activities and attack the pathogens by stealing the nutrition from the pathogens (Wahyuno et al., 2009; Ha, 2010). The application of biological agents in controlling plant diseases is more effective due to their specific activity. It means that this agent reveals better results in its area of origin. Therefore, it is essential to evaluate specific *Trichoderma* species in Berastagi, North Sumatra which can control clubroot disease for having higher and safer cabbage production.

The objectives of this research were to identify specific *Trichoderma* species in three districts in Sumatra Utara and to evaluate the potency *T. harzianum* (both local species from Berastagi and species developed by IVEGRI), to control the disease and then compare them with the application of synthetic fungicide.

## Materials and Methods

The research was conducted in the laboratory and greenhouse in IP2TP, Berastagi, North Sumatera from April–September 2018. The research used cabbage cv. Grand 11 and *Trichoderma* isolates.

A bamboo was cut at both ends. This bamboo was used for *Trichoderma* isolation. The hole inside bamboo was cleaned with running water. Fermented rice was placed into the hole as a medium, and then the hole was closed tightly using plastic wrap. This treated bamboo was put into soil (7–10 cm in depth) for 7–10 days. After 7–10 days, the bamboo was brought to the laboratory to be examined and observed. The fungus growing inside the bamboo was assessed by characterizing its morphology, such as colony color and shape. Those fungi possessing similarities with *Trichoderma* species were then cultured in PDA media in Petri dishes. When *Trichoderma* species were identified, they were re-cultured in Sabouraud Dextrose Agar (SDA) and Sabouraud Yeast Maltose (SYM).

The screened *Trichoderma* species were

propagated in the laboratory on Sabouraud Dextrose Agar (SDA) with conidial density  $10^7$  conidia/ml and then were transferred into a glass jar containing 50 ml of Sabouraud Yeast Maltose (SYM) with the *Trichoderma* on the tip of the glass jar (Misrah & Khan, 2015). The jar was then covered with aluminum foil and was centrifuged at 150 rpm for 9 hours and incubated for 3 days. After that, the fungi were propagated in rice and cracked corn media. The rice and corn had to be washed and boiled for 10 minutes. Each 250 g of the media was placed into a plastic bag. The bamboed pipe was installed on the tip of the plastic bag, and the pipe was covered with sterile cotton, and autoclaved for 1 hour at 121°C. The substrates were then removed and cooled for  $\pm 12$  hours. Propagated *Trichoderma* were put into 3-day-old SYM, and poured into plastic bags containing corn substrate. These bags were incubated for 14 days and were observed every two days. After 14 days, the culture resulting from each substrate was harvested, ground, and saved in the refrigerator to be used in the research (Mishra & Khan, 2015).

The inoculum of *P. brassicae* was obtained from infected cabbage. The infected roots were blended into suspension. The suspensions with conidial density  $10^7$  were then applied to the sterilized soil (Mishra & Khan). These experiments were divided into two stages, where at first stage, all isolates of *Trichoderma* species from Berastagi were examined by applying them into infected soil (sterilized soil treated with *P. brassicae* suspensions) to find the best *Trichoderma* species. After the best species found, we continued to the second stage where the best local *Trichoderma* species (*T. harzianum*) is needed to be re-examined and to be compared with *T. harzianum* obtained from IVEGRI and synthetic fungicide of Nebijin by applying it to infected topsoil and manure (4:1) and was uniformly applied into the media twice, in the seedling and the replanting cabbage and then covered it with soil ( $\pm 1$  cm). The experiments were arranged using Completely Randomized Design (CRD) with 8 treatments: C1 (control-without *Trichoderma*), C2 (*T. harzianum* IVEGRI obtained from corn substrate 2 g/polybag), C3 (*T. harzianum* IVEGRI obtained from rice substrate 2 g/polybag), C4 (*T. harzianum* IVEGRI obtained from corn substrate 4 g/polybag), C5 (*T. harzianum* IVEGRI obtained from rice substrate 4 g/polybag), C6 (local *T. harzianum* obtained from corn substrate 2 g/polybag), C7 (local *T.*

*harzianum* obtained from corn substrate 4 g/polybag) and C8 (synthetic fungicide Nebijin 2 gr per experiment), with four replications. Each treatment consists of 10 plant polybags (size 2.5 kg). The total was 320 test plants.

Parameters observed where Disease Incidence was calculated using Townsend and Heuberger Index (Yudiarti, 2007):

$$DSI = \frac{a}{b} \times 100\%$$

Where: DI = Disease Incidence, a = number of plants infested and b = total number of plants observed.

The severity of clubroot was calculated using the Townsend and Heuberger Index (Yudiarti, 2007) as follows:

$$DSI = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Where: DSI = Disease Severity Index, n = number of infected plants at score v, v = disease score, Z = maximum disease score and N = total number of plants observed.

The disease severity was also assessed by visually estimating the degree of gall development on the lateral and main root system, by harvesting some plants examples, using 0-3 disease score given by Kuginuki et al. (1999), where score 0 = no symptoms of galling, score 1 = small galls on  $<1/3$  of roots, score 2 = moderate/ medium galls on  $1/3$ - $2/3$  of roots and score 3 = severe/ large galls on  $>2/3$  of roots. Analysis of Variance (F Test) was performed, and means were separated using Least Significant Difference (LSD) at probability level 5%.

## Results and Discussions

**Characteristics of *Trichoderma* spp.** The results given in Table 1 indicated different characteristics of *Trichoderma* species observed. The results in Table 1 revealed that there were 7 isolates (D1, D2, Be.1, Be.2, Be.3, M1, and M2) of *Trichoderma* found in three different districts. These isolates were inoculated in PDA media and were propagated for 7 days. These colonies expressed similar colors at 2 and 7 days after application (white and dark green). They were also uniform in round shape (Gupta & Sharma, 2013; Kusmawanto et al., 2022; Agnihotri et al., 2023) and the development of hyphae was slow. Previous research provided findings that *Trichoderma* colony exhibited different colors

during their development, starting with white color, light green, and dark green on the last day of observation (day 7<sup>th</sup>) (Syahputra et al., 2017; Nichols et al., 2018; Sanna et al., 2022; Yadav et al., 2022). The microscopic observation was carried out to examine the size and shape of its conidia, conidiophore, phialides, and hyphae, according to the identification book by Watanabe (2018).

Results in Table 2 clearly showed that the three species possessed different conidiophores and phialides, and only two species revealed the similar shape of conidia, *T. harzianum* and *T.*

*koningii*. This finding is in line with the

research of Gusnawaty et al. (2014), where species *T. harzianum* has erect and branched conidiophore, short and thick phialides.

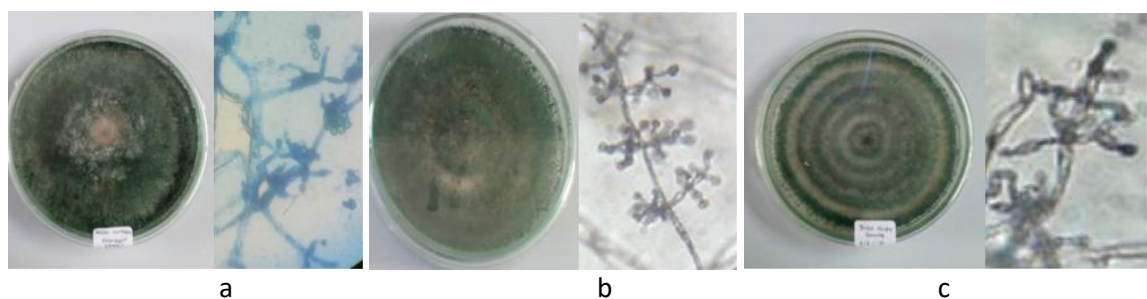
The color is green at 7 days of development (Figure 1a). *T. koningii* expressed erect and branched conidiophore, tapering phialides, with a thinner conidial wall (Figure 1b). *T. viride* demonstrated branched conidiophore, short and thick vertical phialides with the spore balls form at the tip of it, and the color is green in culture (Figure 1c).

**Table 1. Colony development of different *Trichoderma* isolates from three districts**

Location (district)	Isolate Codes	Color of the colony after being planted into PDA (days)			Colony shape
		1	4	7	
Dolat Rayat	D1	White	Light green	Dark green	Round
	D2	White	Light green	Dark green	Round
Berastagi	Be.1	White	Light green	Dark green	Round
	Be.2	White	Whitish light green	Dark green	Round
	Be.3	White	Light green	Dark green	Round
Merek	M1	White	Whitish light green	Dark green	Round
	M2	White	Light green	Dark green	Round

**Table 2. Microscopic observation of *Trichoderma* isolates from three districts**

No	Species	Isolate	Shape		
			Conidiophore	Phialides	Conidia
1.	<i>T. harzianum</i>	D1, Be.1	Erect, branched	Short and thick	Ovate
2.	<i>T. koningii</i>	D2, M2, Be.3	Erect, branched	Tapering towards apex	Ovate
3.	<i>T. viride</i>	M1, Be.2	Branched	Short, thick, vertically arranged	Ovate



**Figure 1. Macroscopic and microscopic appearance of three *Trichoderma* isolates, namely (a) *T. harzianum*, (b) *T. koningii*, (c) *T. viride***

**Table 3. The effect of different *Trichoderma* isolates application on the clubroot disease incidence and severity**

Treatment	Disease incidence (%)	Disease severity (%)
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Tarigan R, Hutabarat RC, Karo BBr, Sembiring P, Napitupulu D, Supardi, Wicaksono RC, Jamaluddin, Setiawati W, Hasyim A. 2024. A comparison of synthetic fungicide and *Trichoderma* spp. applications against clubroot disease on cabbage. Jurnal Kultivasi, 23(1): 91-100

Jo = Control	100 d	100 d
J1 = <i>T. harzianum</i> from Dolat Rayat	30.50 b	10.30 ab
J2 = <i>T. koningii</i> from Dolat Rayat	45.55 bc	28.55 bc
J3 = <i>T. harzianum</i> from Berastagi	0.00 a	0.00 a
J4 = <i>T. koningii</i> from Berastagi	47.00 bc	37.10 bc
J5 = <i>T. viride</i> from Berastagi	40.50 bc	25.10 b
J6 = <i>Trichoderma koningii</i> from Merek	75.00 c	45.50 c
J7 = <i>Trichoderma viride</i> from Merek	57.50 bc	38.25bc
<b>Coefficient of variance</b>	<b>12.42</b>	<b>10.89</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 %.

**Table 4. The effect of different *Trichoderma* isolates and synthetic fungicide application on the clubroot disease incidence from 1-10 weeks after application**

Treatment	Disease Incidence (%)									
	1	2	3	4	5	6	7	8	9	10
	WAA	WAA	WAA	WAA	WAA	WAA	WAA	WAA	WAA	WAA
C1 (control, without <i>Trichoderma</i> )	0 a	0 a	0 a	10 b	20 b	30 c	40 d	50d	70 f	90 e
C2 ( <i>T. harzianum</i> IVEGRI obtained from corn substrate 2 g/ polybag)	0 a	0 a	0 a	0 a	0 a	10 b	20 c	30 b	40 c	40 c
C3 ( <i>T. harzianum</i> IVEGRI obtained from rice substrate 2 g/ polybag)	0 a	0 a	0 a	0 a	0 a	10 b	20 c	30 b	50 d	60 d
C4 ( <i>T. harzianum</i> IVEGRI obtained from corn substrate 4 g/ polybag)	0 a	0 a	0 a	0 a	0a	0 a	10 b	20 b	20 b	30 ab
C5 ( <i>T. harzianum</i> IVEGRI obtained from rice substrate 4 g/ polybag)	0 a	0 a	0 a	0 a	0a	0 a	10 b	20 b	30 b	40 c
C6 ( <i>T. harzianum</i> Berastagi obtained from corn substrate 2 g/ polybag)	0 a	0 a	0 a	0 a	0 a	0 a	10 b	20 b	30 b	30 b
C7 ( <i>T. harzianum</i> Berastagi obtained from corn substrate 4 g/ polybag)	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 o	0 a	0 a
C8 (synthetic fungicide Nebijin)	0 a	0 a	0 a	0 a	10 b	10 b	20 c	30 c	40 c	40 c
<b>Coefficient of Variance (%)</b>	<b>0</b>	<b>0</b>	<b>0</b>	11.77	15.22	16.06	17.07	19.55	22.02	25.78

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 %

*Trichoderma* spp. have been proven to control soil-borne fungal pathogens. The results of ANOVA indicated that *T. harzianum* significantly reduced clubroot disease compared to other *Trichoderma* species (Table 3).

From the results given in Table 3, it indicated that *T. harzianum* obtained from the Berastagi District exhibited the best result (0% disease incidence and 0% disease severity), followed by species *T. harzianum* obtained from Dolat Raya (30.50% disease incidence and 10.30% disease severity). In comparison, the highest disease incidence and severity occurred in plants

treated with *T. koningii* from the Merek District (75.00% and 45.50%). It can be assured that specific *Trichoderma* obtained from its origin place demonstrated better development and ability due to their adaptation to the place, resulting in rapid control of *P. brassicae*.

*T. harzianum* species also grew faster than the other two species, enabling it to degrade the cell wall of the host-pathogen faster. Chamzurni et al. (2013) reported that *T. harzianum* performed better than *T. koningii* in controlling *Rhizoctonia solani* in chilli plant as 75% of chilli seeds possessed better development. The excellent



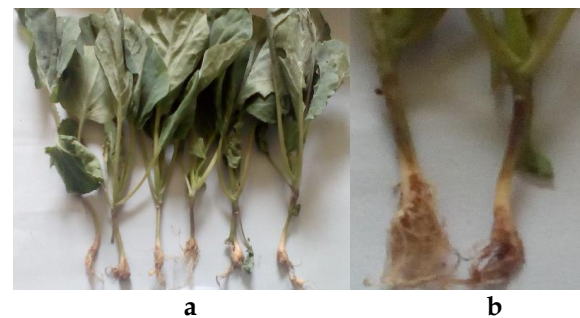
ability of local *T. harzianum* obtained from Berastagi in controlling clubroot disease (Table 3) has allowed this antagonist to be compared with *T. harzianum* developed by IVEGRI in all experiments and also to be compared with the use of synthetic fungicide (Table 4)

The results described that the application of *T. harzianum* both from Berastagi and IVEGRI significantly suppressed the growth of clubroot disease in cabbage plants. The application of the antagonist gave significant results on clubroot disease control. The lowest disease incidence has been found in plants treated with 4 g local *T. harzianum* obtained from corn substrate (C7), resulting in 0% intensity, followed by the application of 4 g *T. harzianum* IVEGRI obtained from corn substrate (C4) with disease intensity of 30%. The highest intensity was exhibited by control (C1, without *Trichoderma*) with 90% damage at 10 weeks after application (WAA), followed by the application of 2 g *T. harzianum* IVEGRI obtained from rice substrate (C3) (60%). These findings indicated that substrates play an important role in antagonist ability to suppress the pathogen development, resulting in low severity (Soenartiningih et al., 2014).

In addition, the ability of antagonists in suppressing the pathogen growth was influenced

by nutrient sufficiency provided from the planting media such as carbon, nitrogen, carbohydrate, and glucose (Yulia et al., 2017), which enables them to compete for the growth space, inhibiting pathogen from colonizing and slowly dying (Amaria et al., 2013). Fast colonization by antagonists has disabled pathogens to grow and widened the colonization.

The application of antagonist *Trichoderma* spp. has influenced the performance and also the appearance of its roots (Figure 2).



**Figure 2.** The comparison between (a) untreated plant and (b) treated ones. The used *Trichoderma* was *T. harzianum* from Berastagi, Sumatra Utara

**Table 5.** The effect of different *Trichoderma* isolates and synthetic fungicide application on the clubroot disease severity and root fresh weight

Treatment	Disease Severity (%)	Root fresh weight (g)
C1 (control, without <i>Trichoderma</i> )	85.65 e	43.48 e
C2 ( <i>T. harzianum</i> IVEGRI obtained from corn substrate 2 g/polybag)	37.77 c	29.61 b
C3 ( <i>T. harzianum</i> IVEGRI obtained from rice substrate 2 g/polybag)	79.11c	37.49 d
C4 ( <i>T. harzianum</i> IVEGRI obtained from corn substrate 4 g/polybag)	28.52 b	19.70 b
C5 ( <i>T. harzianum</i> IVEGRI obtained from rice substrate 4 g/polybag)	39.11	32.55 c
C6 ( <i>T. harzianum</i> Berastagi obtained from corn substrate 2 g/polybag)	29.02 b	20.02 b
C7 ( <i>T. harzianum</i> Berastagi obtained from corn substrate 4 g/polybag)	0.00 a	7.90 a
C8 (synthetic fungicide Nebijin)	45.19 d	35.2 c
<b>Coefficient of variance</b>	<b>15.85</b>	<b>23.82</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 %

The application of 4 g local *Trichoderma*

obtained corn substrate caused no attack to the plants (0%) with the lowest root weight (7.90 g), followed by the application of 4 g *Trichoderma*

obtained from corn substrate (28.52 % and 19.70g). The untreated plants have possessed the highest disease severity (85.65%), which resulted in the highest root weight (63.48g) (Table 5). It indicated that the attack of *P. brassicae* has a positive correlation to root weight. Not only suppressed the growth of *P. brassicae*, these beneficial fungi also have reduced the diseases caused by not only bacterial and fungal pathogens, for instance *Ralstonia solanacearum* in tomato and brinjal (Guo et al, 2021; Qulsum et al., 2023), *Erwinia carotovora* in potato and cabbage (Le et al., 2020; Sulaiman et al., 2020), *Phytophthora* spp. in pepper and chestnut (Timila & Manandhar, 2020; Frascella et al., 2022), *Fusarium* spp. in asparagus, cereal, tomato and chili (Anjum et al., 2020; Hasan et al., 2020; Modrzweska et al., 2022; Brizuela et al., 2023), but also nematodes (Ibrahim et al., 2020; Javeed et al., 2021; Yan et al., 2021; Nafady et al., 2022, Kassam et al., 2023). Dwiastuti (2016) reported that higher disease severity cause d higher swollen roots, where these swollen roots contributed to higher weight of roots. *Trichoderma* suppress the invasion of pathogen by releasing enzymes  $\beta$ -1,3 glucanase, chitinase, and cellulase to inhibit cell wall permeability of pathogen's, causing pathogen mortality.

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## Conclusion

There were three species of *Trichoderma* spp. found in three different districts in Berastagi Regency, namely, *T. harzianum*, *T. viride* and *T. koningii*. The application of 4 g local (Berastagi) *Trichoderma* obtained from corn substrate has significantly suppressed clubroot disease incidence and severity in cabbage plants by 0%.

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## The effect of fruit maturation stage on seed germination of *iaa9-3* and *iaa9-5* tomato mutants

**Abstract.** One of the internal factors that play an important role in seed quality is the level of the phytohormone auxin. Efforts to increase auxin in seeds include developing new varieties with increased indigenous auxin, such as those found in mutant tomatoes from Micro-Tom, namely *iaa9-3* and *iaa9-5*. This research was conducted to determine the germination response of *iaa9-3* and *iaa9-5* mutant tomato seeds at different levels of the fruit maturation stage. The research was carried out at the Seed Technology Laboratory, Faculty of Agriculture, Padjadjaran University, and the Seed Testing Laboratory of the Center for Standard Testing of Vegetable Plant Instruments. The response design used was a completely randomized factorial design consisting of two factors and repeated three times. The first factor was the *iaa9-3*, *iaa9-5*, and Wild-Type Micro-Tom (WT-MT) mutants as controls. The second factor is the fruit maturation stage, which consists of Breaker, Pink, and Red. The research results showed that there was an interaction between genotype and fruit maturation stage on the parameters of germination, growth simultaneity, and hypocotyl length. The harvest stage for tomatoes to produce normal strong seed germination was the pink stage in all tomato genotypes tested. The best germination rate was shown by WTMT seeds at the pink stage, genotype *iaa9-3* at the red stage, and genotype *iaa9-5* at the red or pink stage. The effect of fruit maturity stage on the synchronization of sprout growth was relatively not significantly different in mutant tomatoes, but it had an effect in WTMT tomatoes, namely the best pink stage.

**Keywords:** Auxin • Breaker • Germination rate • Micro-tom

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## Introduction

Optimal plant growth and development is a success in the cultivation process. Among the factors that are very important in the process of accelerating plant growth and development are phytohormones. Phytohormones function to stimulate growth and regulate physiological processes that occur in plants (Sukmadi, 2013). One of the phytohormones that play an important role in the process of plant growth and development is auxin.

Auxin has an important role in improving seed quality. Auxin acts as a key regulator to modulate cellular processes in seed development (Cao et al., 2020). According to Zhao & Zhong (2013), exogenous administration of indole acetic acid (IAA) affects the early stages of seed germination and stimulates seed germination. This is thought to be related to the repair of membranes and seed respiration that occurs due to the administration of exogenous auxin.

*iaa9-3* and *iaa9-5* tomatoes are two mutant tomatoes, from the clay parent Micro-Tom (WTMT) which experienced mutations due to the mutagen of ethyl methyl sulfonate (Saito et al., 2011). *IAA9* is a family of Aux/IAA genes that plays a role in suppressing transcription of the endogenous auxin signal pathway, i.e., IAA (Guilfoyle & Hagen, 2007). The effect of mutations in *IAA9* will trigger a different auxin response from its parents (Wang et al., 2005).

Increasing seed quality with the presence of auxin is also influenced by the physiological ripening conditions of the seeds. According to Rusmin et al., (2007) improving the quality of seeds is influenced by the physiological maturation conditions of the seeds at the right time of harvest. Appropriate physiological ripening conditions result in maximum dry weight and seed vigor. So if harvesting is not in the right conditions, the seeds produced tend to be of suboptimal quality. The stage of fruit maturation greatly influences seed vigor. When fruit is harvested too young, the quality of the seeds produced tends to have low physiological potential. This condition is related to immature seeds due to suboptimal seed filling in the fruit (Tetteh et al., 2018).

In WTMT tomatoes themselves, there are several stages of fruit development. According to Wang et al., (2005), the stages of development

of Micro-Tom tomato fruit are divided into; early light green (EIM), light green (IM), dark green (MG), breaker (BR), orange (OR), early red (ER), red (R), and mature red (RR). Unfortunately, there has been no study of differences in fruit maturation stages on the quality of WTMT mutant tomato seeds. This research aimed to determine the germination response of *iaa9-3* and *iaa9-5* mutant tomato seeds at different levels of fruit maturation.

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## Materials and Methods

This research was conducted at (i) the Seed Laboratory of the Faculty of Agriculture, Universitas Padjadjaran, located in Jatinangor, Sumedang Regency, West Java, and (ii) the Seed Testing Laboratory of the Vegetable Crops Research Institute in Lembang, Bandung Regency, West Java.

The experiments utilized tomato planting materials, namely *iaa9-3*, *iaa9-5*, and their respective wild parents (WT-MT) as controls. The experimental design employed a factorial completely randomized design with two treatment factors. The first factor comprised the mutant lines, i.e., *iaa9-3*, *iaa9-5*, and WTMT (control), while the second factor involved the fruit maturation levels of the breaker, pink, and red-stage seeds (Mubarok et al., 2023).

**Seeding Preparation.** Seeding was conducted using a husk charcoal and cocopeat planting medium in a 1:1 ratio in a seed tray. Subsequently, tomato seeds that were 15-20 days old or had formed 4-5 leaves were transferred to planting pots. Plant maintenance included replanting, watering, fertilizing, and controlling pests and diseases.

**Seed Quality Testing.** Tomato fruits are harvested at maturation stages including breaker, pink, and red. Subsequently, the seeds undergo a seed quality test consisting of the following procedures:

- (a) Calculation of the percentage of normal, abnormal, and dead sprouts from the beginning to the end of a 14-day observation period.
- (b) Measurement of hypocotyl length using a ruler, from the base to the point of the cotyledon stalk, expressed in centimeters (cm).
- (c) Assessment of germination capacity by observing normal tomato germination.

Normal sprouts exhibit essential structures such as primary roots, coleoptiles, and well-developed plumules. The calculation of normal germination can be performed using the formula provided by Sutopo (2010).

- (d) Assessment of the simultaneity of seed growing used to determine the percentage of seeds capable of normal germination under optimal conditions by the 7<sup>th</sup> day (first-day count).

**Data analysis.** Data were analyzed using a two-way analysis of variance (ANOVA). Further tests were carried out using the Duncan test at a significance level of 5%.

## Results And Discussion

### Percentage of Strong Normal Sprouts, Weak Normal Sprouts, Abnormal, and Dead Sprouts.

The results of the observations showed that there were seeds that experienced normal strong, normal weak, abnormal, and dead germination. Normal strong sprouts are characterized by good root and hypocotyl growth, and the seeds can separate from the cotyledons. Meanwhile, normal weak sprouts are not separated from the cotyledons, and root and hypocotyl growth tends to be suboptimal (Nuraini et al., 2024). Abnormal sprouts show stunted and unbalanced growth between the roots and hypocotyl. Moreover, the seeds are not able to stand up straight because they are too small. Dead sprouts are caused by fungi and bacteria, which cause the seeds to die and not grow at all.

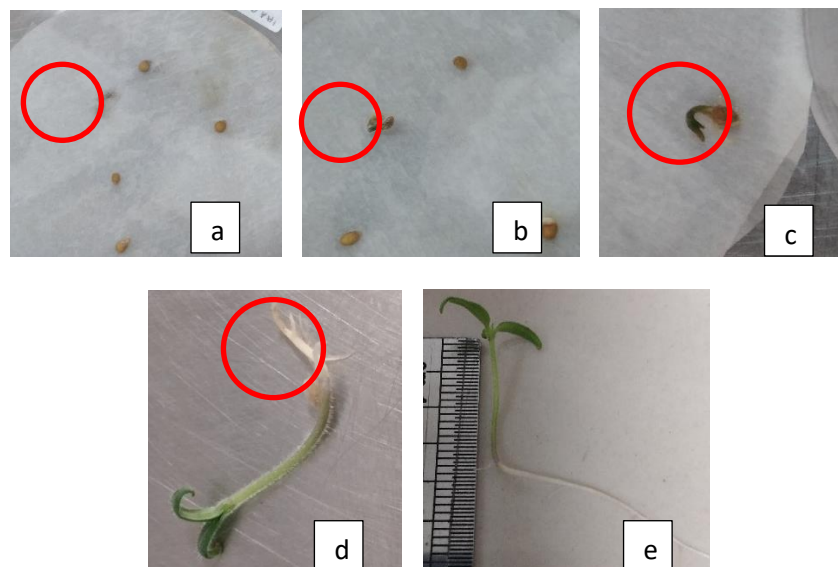
Table 1 shows the percentage of strong normal sprouts, weak normal sprouts, abnormal sprouts, and dead sprouts. During the 14-day germination process, the best percentage of normal strong sprouts was shown in the *iaa9-5* treatment at the maturation level of pink fruit, with an average of 7 sprouts. On average, strong normal sprout development occurred in the pink phase, both in the *iaa9-3*, *iaa9-5*, or WTMT lines. This condition shows that fruit maturation at the pink stage is the optimal phase for seed quality, as at this stage, the seeds are neither too young nor too old. Conversely, in the breaker phase, the seeds are still too young, and in the red phase, the seeds are too old.

This condition aligns with research conducted by Tetteh et al., (2018) regarding the influence of seed maturation level on seed quality, which indicates that seeds that are too young seed derived from green fruit stage had a low germination rate. Moreover, the *iaa9-3* and *iaa9-5* mutants showed different germination results. Even though they were harvested in the breaker and red phases, they still exhibited good germination. This condition may be attributed to the presence of auxin in mutant plants, which affects the ability of seeds to germinate. According to Maemunah & Adelina (2009), in the process of seed metabolism, auxin acts as the main compound. The presence of auxin causes cell division and stimulates the formation of young roots. When auxin is in optimal conditions in the plant, it facilitates the utilization of growth factors such as water, nutrients, and food reserves in the cotyledons by the seeds. The optimal presence of auxin corresponds to the high seed-growing performances.

**Table 1. The mean of strong normal, weak normal, abnormal, and dead sprouts in three tomatoes obtained from different fruit maturation stages**

No	Treatment		Strong Normal	Mean of Sprouts		
	Genotype	Fruit Maturation Stage		Weak Normal	Abnormal	Dead
1	WTMT	Breaker	1.67	2.00	1.33	4.00
2	WTMT	Pink	7.33	5.67	2.67	0.00
3	WTMT	Red	1.33	0.67	0.00	2.67
4	<i>iaa9-3</i>	Breaker	1.67	1.00	1.00	3.67
5	<i>iaa9-3</i>	Pink	5.33	5.33	0.67	3.00
6	<i>iaa9-3</i>	Red	4.33	9.00	0.67	2.00
7	<i>iaa9-5</i>	Breaker	3.33	5.33	1.00	6.33
8	<i>iaa9-5</i>	Pink	7.00	6.00	0.33	3.67
9	<i>iaa9-5</i>	Red	5.00	4.67	0.67	4.33





**Figure 1. Morphological appearance of sprouts: (a) dead sprout as infected by fungus, (b) dead sprout as infected by bacterial pathogen, (c) abnormal sprout, (d) weak normal sprout, (e) strong normal sprout**

These germination conditions indicate an influence between genotype and fruit maturation level. The pink stage is the optimal maturation level because it is neither too young nor too old. Meanwhile, the genotype factor shows an increase in germination ability at the breaker or red stage.

Figure 1 provides the differences between normal, weak, strong, and abnormal sprouts. Weak normal sprouts are characterized by unbalanced growth between the roots and the hypocotyl. Meanwhile, abnormal sprouts tend to be stunted and fail to develop. On the other hand, in normal sprouts, strong root growth and hypocotyl development occur in a balanced manner.

The dead seeds in this study were affected by bacteria and fungi. Seeds affected by fungus can be identified by the fine white threads surrounding them (Figure 1a). In contrast, seeds affected by bacteria show softness and destruction (Figure 1b).

**Germination Rate.** The results of the analysis revealed significant differences and interactions between tomato genotype and fruit maturation stage. In WTMT tomatoes, seed germination at the green or red fruit maturation stage demonstrates a low percentage. This finding corresponds with research by Pradnyawati et al. (2019) on the influence of harvest age on long bean seeds. Seeds harvested too young tend to exhibit suboptimal seed

quality due to many seeds not yet being physiologically mature. Conversely, late harvest diminishes seed quality.

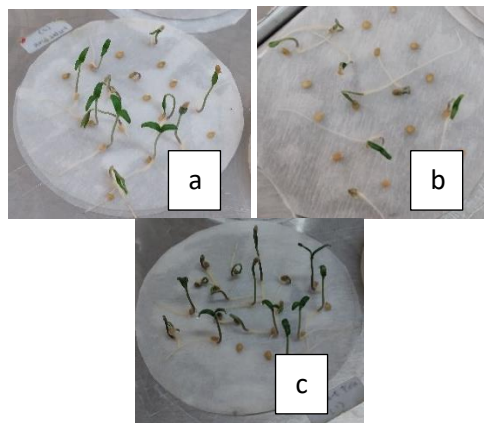
Referring to Table 2, the *iaa9-3* and *iaa9-5* mutant tomatoes exhibit robust germination at both green and red harvest times. This phenomenon may be attributed to an increase in auxin in mutant tomatoes, which affects seed germination. This aligns with research by Adnan et al. (2017) on the application of Auxin to enhance the viability of aged watermelon seeds, resulting in an increased germination percentage. Auxin treatment accelerates sprout growth, leading to the production of vigorous seedlings.

**Table 2. The interaction effect of tomato genotypes and fruit maturation stages on the seed germination rate**

Fruit maturation stages	Genotype		
	WTMT	<i>iaa9-3</i>	<i>iaa9-5</i>
<b>Breaker</b>	6.67a	5.00a	11.67a
	A	A	A
<b>Pink</b>	28.33b	8.33a	25.00b
	B	A	B
<b>Red</b>	14.33a	11.33b	21.67b
	A	A	B

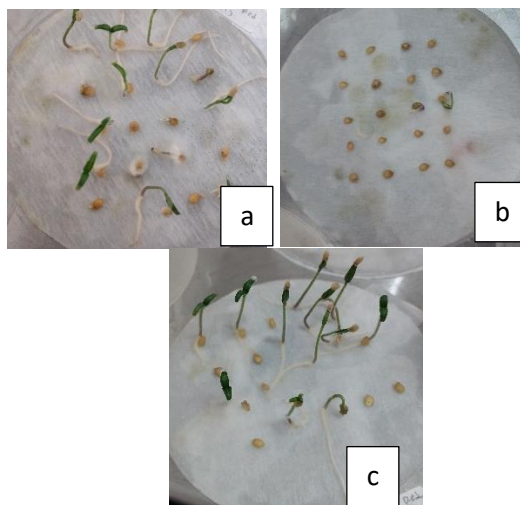
Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.





**Figure 2. Morphological appearance of sprouts at 7 days after germination, obtained from seed in pink fruit maturation stages: (a) WTMT, (b) *iaa9-3*, (c) *iaa9-5*.**

In Figure 2, it can be seen that the germination capacity of the seed obtained from the pink fruit maturation stage at 7 days after sowing was quite good. Germination conditions are much better than the breaker or red phase. This can be seen in Figure 3.



**Figure 3. Morphological appearance of sprouts at 7 days after germination, obtained from seed in red fruit maturation stages: (a) *iaa9-3*, (b) WTMT, and (c) *iaa9-5*.**

The effect of auxin on germination can be seen in Figure 3. Seeds from the WTMT line at the red fruit maturation stage showed low germination, and did not even germinate. However, seeds from genotypes *iaa9-3* and *iaa9-5* showed good germination even though they were taken from red stage fruit.

**The Simultaneity of Seed Growing.** The results of the seed simultaneity test show that there is an interaction between genotype and the level of seed maturation. Based on Table 3, the harvest age of the pink stage in the WTMT line has a fairly good level of seed growth synchrony, followed by the *iaa9-5* line. These results show that the pink stage is the best seed maturation level to produce quality seeds. In the breaker or red stages, results tend to be not very good. This condition can occur due to the existence of optimum food reserves at the maturation level of seeds that are not too young, but also not too old. Because the condition of the seeds is too young, the food reserves in the seeds are not yet optimal. On the other hand, at the maturation level of seeds that are too old, the food reserves contained in the seeds decrease.

**Table 3. The interaction effect of tomato genotypes and fruit maturation stages on the simultaneity of seed growing**

Fruit Maturation Stage	Genotype		
	WTMT	<i>iaa9-3</i>	<i>iaa9-5</i>
Breaker	0.05 a A	0.05 a A	0.08 a A
Pink	0.20 b B	0.03 a A	0.13 a B
Red	0.02 a A	0.03 a A	0.13 a B

Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.

The influence of fruit maturation level is in line with research conducted by Wulananggraeni et al. (2016) regarding the effect of differences in fruit maturation levels in 3 genotypes of cucumber on seed quality. Cucumber seeds that are harvested 18 days after flowering have a good effect on the simultaneous growth of the seeds. Wulananggaraeni et al. (2016) explained that this situation was caused by the condition of the seeds being ripe and not too ripe when harvested. The food reserves contained in the seeds are in optimum condition and come from food deposits resulting from photosynthesis. The influence of maturation level on seed quality was also revealed by Early harvest, before physiological maturation, which results in many seeds not being fully formed and filled

so it will produce seeds of low quality because many of the seeds are wrinkled. Meanwhile, harvesting after physiological maturation will most likely experience harsh conditions on the seeds, resulting in many seeds experiencing a decline in quality before harvest.

**Hypocotyl Length.** The results of the analysis for hypocotyl length at 14 days after sowing showed that there was an interaction between genotype and seed harvest age. The response shown was that the hypocotyl length at the breaker or red harvest age still showed good growth. In contrast, the WT-MT control plants only grew optimally at the pink harvest age (Table 4).

**Table 4. The interaction effect of tomato genotypes and fruit maturation stages on the length of seed hypocotyl**

Fruit Maturation Stage	Genotype		
	WT-MT	<i>iaa9-3</i>	<i>iaa9-5</i>
<b>Breaker</b>	0.57b A	0.79a B	1.04a C
<b>Pink</b>	1.62c C	1.39b A	1.58c B
<b>Red</b>	0.25a A	1.46c C	1.17b B

Notes: Means followed by the same letter in the same column and factor is not significantly different based on Duncan's Multiple Range Test at 5% significance level. Lowercase letters are read vertically and capital letters are read horizontally.

At the breaker stage, the germinated seeds tend to be dwarfed. This situation can be caused by the harvest being too young so that the seeds are not yet at the physiological maturation phase. According to Singkaew et al., (2017) at the green harvest age, the condition of the seed pericarp has not developed optimally and the process of translocating nutrients from fruit to seed does not run optimally. This condition causes too-young harvested seeds are not develop well.

Apart from that, WT-MT tomatoes whose seeds were harvested at the green or breaker harvest age did not germinate optimally and did not even grow. Meanwhile, the *iaa9-3* or *iaa9-5* mutagens showed signs of growth even though they were harvested at different stages. As can be seen in Figure 3 which shows the condition of the hypocotyl length at the breaker stage. In *iaa9-3* mutants and *iaa9-5* mutants, the hypocotyl length is better and more upright, even at the

breaker stage. Meanwhile, as seen in Figure 3, the length of the hypocotyl in WTMT is shorter and tends to be less upright.



**Figure 3. Hypocotyl length of seed obtained from breaker fruit maturation stages: (a) WTMT, (b) *iaa9-5*, (c) *iaa9-3*.**

## Conclusion

There was an interaction between genotype and fruit maturation stage on the parameters of germination, growth simultaneity, and hypocotyl length. The harvest stage for tomatoes to produce normal strong seed germination was the pink stage in all tomato genotypes tested. The best germination rate was shown by WTMT seeds at the pink stage, genotype *iaa9-3* at the red stage, and genotype *iaa9-5* at the red or pink stage. The effect of the fruit maturity stage on the simultaneity of seed growing was relatively not significantly different in mutant tomatoes, but it had an effect in WTMT tomatoes, namely the best pink stage.

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## Additional cocopeat and coconut water improves the seedling growth of robusta coffee

**Abstract.** Robusta coffee production can be succeeded by using high-quality seedlings that have good growth performance. One of several keys to success in seedling production is the choosing best planting medium and providing exogenous growth regulators. These include using cocopeat planting media and giving coconut water. This research aims to determine whether using cocopeat planting media and providing coconut water can increase the growth of robusta coffee seedlings. The experiment used a randomized complete group design with six treatments and four replications; each experimental unit consisted of three robusta coffee seedlings. The six tested treatments were control (topsoil 100%); cocopeat 50% + topsoil 50%; cocopeat 100%; topsoil 100% + coconut water 200 mL; cocopeat 50% + topsoil 50% + coconut water 200 mL; and cocopeat 100% + coconut water 200 mL. This study indicated that cocopeat and coconut water could increase the growth of robusta coffee seedlings. The composition of cocopeat 100% and the combination of topsoil 50% + cocopeat 50% had an improvement effect on the growth of robusta coffee seedlings.

**Keywords:** Cocopeat · Coconut water · Growth regulator · Nursery · Robusta coffee

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## Introduction

Robusta coffee is one of the commodities with strategic value in empowering the people's economy (Directorate General of Plantations, 2012). Robusta coffee is currently the dominant type of coffee plantation in Indonesia. One of the advantages of robusta coffee compared to Arabica coffee is that it is more resistant to pests and diseases and has a flavor that is generally not owned by other coffee producers (Sari & Ibrahim, 2020)

Indonesian Plantation Statistics (Directorate General of Plantations, 2022), mentioned robusta coffee production data in 2019 with an area of 860,438 ha producing robusta coffee of 538,171 tons. In 2020, the area and production of robusta coffee increased to 860,777 ha, producing 539,856 tons. Furthermore, in 2021 it became 870,616 ha, producing 560,466 tons. Robusta coffee area decreased in 2022 to 868,785, but production increased to 572,570 tons.

As the area of robusta coffee plantations increases, robusta coffee production also increases. Robusta coffee production can be increased with good plant growth. Plant growth starts from seedlings. The nursery is the initial stage of plant management that determines the sustainability of cultivation. It begins with planting preparation by selecting superior seeds, planting media, and maintenance, including providing growth regulators for optimal seedling growth.

Coffee nurseries generally use topsoil as a planting medium. The use of topsoil in large quantities can harm the environmental balance, and the use of topsoil as a nursery planting medium should be limited to avoid the negative impact of massive topsoil extraction (International Tropical Timber Organization, 2006). Therefore, it is necessary to find other media that are available in large quantities and easy to find but can still support the growth of coffee seedlings well. One of these media is cocopeat. Cocopeat is a planting medium made from coconut fiber. Cocopeat planting media is expected to be used as a planting medium to replace topsoil and a step in utilizing coconut plant waste. Cocopeat can bind and store water strongly and contains essential nutrients. Media

directly affect root system development and function. A good-quality growing medium provides sufficient anchorage or support to the plant and serves as a reservoir for nutrients and water (Krishnapillai et al., 2020).

Besides planting media, other efforts to obtain good coffee seedling growth require nursery-stage maintenance activities, including growth regulators. Growth regulators are non-nutrient organic compounds that can support the plant growth process. One of the PGRs widely used to stimulate growth in the vegetative period is cytokinin. Cytokinins function for organ formation, delaying aging, spurring the development of side buds of dicotyledonous plants, spurring chloroplast development, and chlorophyll synthesis (Amsyahputra et al., 2016). One of the natural substances in the cytokinin group is coconut water. Coconut water can be utilized naturally because it contains hormones that spur plant growth.

The results of Singchal's research (2019), the composition of planting media consisting of 50% soil and 50% cocopeat could increase plant height and stem diameter growth in the early growth of Arabica coffee seedlings. The results of research by Rosianty et al. (2021) also show that the use of cocopeat combined with soil in a ratio of 1: 1 was the best composition for the growth of stem diameter of *Eucalyptus pellita* F. Muell.

According to Mergiana et al. (2021), Applying coconut water 200 mL/plant has the best effect on the number and width of grapevine leaves. According to Darlina et al. (2016), watering coconut water 200 mL / L of water produced the highest number of leaves, wet weight, and dry weight in pepper plants. The research results by Kasi et al. (2021) also show that applying coconut water 200ml / L was the best concentration for the growth of apple seedlings.

Based on the previous description, Cocopeat has good nutrient content, so it can replace topsoil as a planting medium, and providing coconut water can help supplement nutrients that are good for plant growth. Therefore, this study was conducted to determine the effect of additional cocopeat and coconut water on the growth of robusta coffee seedlings.

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## Material and Method

The experiment was conducted from March to May 2023 in the Ciparanje experimental garden, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java, at an altitude of 750 m above sea level, with soil order Inceptisols, and rainfall type C based on Schmidt and Ferguson (1951) classification.

The tools used in this research were a polybag 35 cm x 35 cm, hoe, machete, stationery, meter, ruler, vernier, bucket, chlorophyll meter CCM-200 Plus type, and camera. The materials used in this study were 9-month-old robusta coffee seedlings of BP 939 variety, cocopeat planting media, topsoil, coconut water, and water.

The analysis of the planting media carried out is of topsoil and cocopeat. Topsoil planting media was analyzed at the Laboratory of Soil Fertility and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran. The results are secondary data from the soil analysis results in the Ciparanje experimental garden, Faculty of Agriculture Unpad.

Analysis of cocopeat planting media was conducted at the Soil Chemistry and Plant Nutrition Laboratory, Faculty of Agriculture, Padjadjaran University. The materials tested were cocopeat samples used as planting media in this study. The analysis was conducted to determine the content of elements present in the planting media used, such as pH, N, P, K, C, and moisture content was carried out at the Laboratory of Soil Fertility and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran.

Analysis of coconut water content was carried out at the ICBB Laboratory of PT Biodiversity Biotechnology Indonesia.

Calculating leaf area is done using the Image J application by photographing all leaves, changing the leaf color to gray, and then clicking on the leaf object to be measured using the wand tool. The measurement results will be displayed through measures on the analysis menu (Susanti & Safrina, 2018). Chlorophyll content index was measured in plant leaves using a CCM-200 plus chlorophyll meter and expressed in Chlorophyll Content Index (CCI) units. The number of leaves, leaf area, plant height, stem diameter, and chlorophyll content index were observed from 4 weeks after planting to 12 weeks after planting at two-week intervals.

The experimental method used was a randomized block design (RBD) with eight treatment combinations. Each treatment was repeated 4 times so that there were 24 experimental units. Each experimental unit contained three plants, the total of which was 72.

The treatments are arranged as follows: A = control (topsoil 100%), B = cocopeat 50% dan topsoil 50%, C = cocopeat 100%, D = topsoil 100% + coconut water 200 mL, E = cocopeat 50% dan top soil 50% + coconut water 200 mL F = cocopeat 100% + coconut water 200 mL. Data were analyzed using analysis of variance (ANOVA) for the F test at a 95% confidence level. Duncan's multiple range test was conducted at a 95% confidence level if there were differences between treatments.

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## Result and Discussion

**Environmental observation.** Environmental observations, including weather, are also useful for identifying climatic conditions that affect the development and maintenance of coffee nurseries to be optimized. temperature and rainfall data were obtained from the Ciparanje weather station, Faculty of Agriculture, Universitas Padjadjaran. The average temperature during the study ranged from 22.7°C-23.7°C, with the highest temperature of 23.7°C in April 2023 and the lowest at 22.7°C in February 2023. This is the optimal temperature for robusta coffee growth, which ranges from 22-25°C. (Djaenudin et al., 2011). Air temperatures of more than 25oC cause the rate of photosynthesis to decrease, and the leaves become damaged, starting with chlorosis, and vice versa at temperatures that are too low (less than 20°C) can disrupt the physiological activities of the leaves so that the color turns yellowish starting from the edges of the leaves. (Pujianto, 1999).

The average rainfall during the study ranged from 105.50-238.0mm/month, classified as a wet month. The highest rainfall occurred in April 2023 at 238 mm/month, while the lowest occurred in February 2023 at 105.50 mm/month. This is by the optimal rainfall for Robusta coffee growth, ranging from 100-250 mm/month to around 2000-3000 mm/year (Djaenudin et al., 2011).

**Analysis of Soil, cocopeat, and coconut water.** The results of the soil analysis. The test



results showed that the pH of the topsoil planting media included slightly acidic criteria, low N-total, and K<sub>2</sub>O. The pH level and nutrient content of N, P, and K contained in cocopeat planting media are better than in topsoil planting media. C-organic content in cocopeat showed higher results.

**Table 1. Average Daily Temperature, Relative Humidity, Total Rainfall, Evaporation, and Sunlight Intensity during Research Period**

Month	Daily temperature (°C)	Relative Humidity (%)	Total Rainfall (mm/month)	Evaporation (mm/month)	Light intensity (%)
February	22.70	91.00	105.50	2.80	26.00
March	22.90	88.00	132.50	3.30	74.00
April	23.70	88.00	238.00	3.10	79.00
May	23.00	88.00	139.00	3.70	75.00

Source: Weather Station of Ciparanje Experimental Farm, Faculty of Agriculture Unpad, 2023

**Table 2. Soil Analysis**

No	Parameter	Unit	Result
1	pH: H <sub>2</sub> O	-	5.96
2	N-total	%	0.12
3	P <sub>2</sub> O <sub>5</sub> HCl 25%	mg/100g	60.65
4	P <sub>2</sub> O <sub>5</sub>	ppm	10.70
5	K <sub>2</sub> O HCl 25%	mg/100g	11.60
6	K-dd	cmol.kg <sup>-1</sup>	1.48

Source: Laboratory of Soil Fertility and Plant Nutrition, Faculty of Agriculture, Universitas Padjadjaran, 2023

The results of cocopeat planting media (Table 3) showed that the pH of the topsoil planting media included slightly acidic criteria, low N-total, and K<sub>2</sub>O. The pH level and nutrient content of N, P, and K contained in the cocopeat planting media are better than the topsoil planting media. The C-organic content of cocopeat showed higher results. Based on the topsoil and cocopeat analysis test results, it can be concluded that cocopeat can be used as an alternative planting media mixture.

The analysis of coconut water showed that total N was 0.01%, P<sub>2</sub>O<sub>5</sub> was 0.03%, K<sub>2</sub>O was 0.21%, calcium (Ca) was 200.00 mg/L, magnesium (Mg) was 99.50 mg/L, pH was 3.8, sodium (Na) was 365.00 mg/L, cytokinin in the form of kinetin was 17.552 mg/L, and cytokinin in the form of zeatin was 14.973 mg/L (Table 4).

The results of several studies show that coconut water is rich in potassium, minerals including calcium (Ca), sodium (Na), magnesium (Mg), ferrum (Fe), cuprum (Cu), sulfur (S), sugar, and protein. The mineral content is the growth hormone that plants need. In addition to being rich in minerals, coconut water also contains two natural hormones, auxin, and cytokinin, which play a role in supporting cell division (Tiwery, 2014).

**Table 3. Analysis of Cocopeat Planting Media**

No	Parameters	Unit	Result
1	Organic - C	%	56.02
2	C/N	-	72.75
3	Moisture Content	%	80.74
4	pH	-	7.72
5	N	%	0.77
6	P <sub>2</sub> O <sub>5</sub>	%	23.31
7	K <sub>2</sub> O	%	3.92

Source: Laboratory of Soil Fertility and Plant Nutrition, Faculty of Agriculture, Padjadjaran University, 2023

**Table 4. Analysis of coconut water**

No	Parameters	Unit	result
1	N Total	%	0.01
2	P <sub>2</sub> O <sub>5</sub> Total	%	0.03
3	K <sub>2</sub> O Total	%	0.21
4	Calcium (Ca)	mg/L	200.00
5	Magnesium (Mg)	mg/L	99.50
6	pH	-	3.8
7	Sodium (Na)	mg/L	365.00
8	cytokinin		
	Kinetin	mg/L	17.552
	Zeatin	mg/L	14.973

Source: ICBB Laboratory - PT Biodiversitas Biotechnology Indonesia, 2023

**Number of Leaves.** Based on the results of the F test at the 95% confidence level, it shows that treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) is a treatment that gives a better effect than the other treatments, but not significantly different from treatment B (cocopeat 50% and topsoil 50%), treatment D (topsoil 100% + coconut water 200 mL), and treatment F (cocopeat + coconut water 200 mL) at the age of 4 weeks after planting. At the age of 6-12 weeks after planting, treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) was significantly different from treatment A (control), treatment B (cocopeat 50% and topsoil 50%), and treatment C (cocopeat 100%) (Table 5).

**Table 5. Effect of the Combination of Planting Media and Coconut Water on the Number of Leaves of Robusta Coffee Seedlings at 4 - 12 WAP**

Treatment	Number of Leaves				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
A = control (topsoil 100%)	7.67 a	8.08 a	9.00 a	9.17 a	9.50 a
B = cocopeat 50% + topsoil 50%	8.50 ab	8.58 a	9.67 a	10.08 a	10.75 a
C = cocopeat 100%	7.75 a	8.09 a	8.92 a	9.25 a	10.25 a
D = top soil 100% + coconut wat 200 mL	9.67 ab	9.84 ab	11.09 ab	11.25 ab	12.42 ab
E = cocopeat 50% + topsoil 50% + coconut water 200 mL	10.50 b	11.84 b	12.67 b	13.17 b	14.67 b
F = cocopeat 100% + coconut water 200 mL	9.34 ab	9.75 ab	10.25 ab	10.25 b	12.08 ab

Note : WAP = Week after planting. Mean followed by different letters within the similar column was significantly different based on Duncan multiple range test at the 5% level.

**Table 6. Effect of the Combination of Planting Media and Coconut Water on the Leaf Area of Robusta Coffee Seedlings at 4 - 12 WAP**

treatment	Leaf area index				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
A = control (topsoil 100%)	35.08 a	34.87 a	40.13 a	43.52 a	46.20 a
B = cocopeat 50% + topsoil 50%	44.49 b	44.66 c	50.83 b	49.94 ab	55.04 b
C = cocopeat 100%	65.44 c	64.16 d	80.51 c	84.66 c	88.17 c
D = top soil 100% + coconut water 200 mL	38.16 a	37.44 ab	44.58 ab	45.67 ab	50.38 ab
E = cocopeat 50% + topsoil 50% + coconut water 200 mL	45.33 b	42.01 bc	47.93 b	50.70 b	54.61 b
F = cocopeat 100% + coconut water 200 mL	67.28 c	65.92 d	76.51 c	84.44 c	82.83 c

Note : WAP = Week after planting. Mean followed by different letters within the similar column was significantly different based on Duncan multiple range test at the 5% level.

This shows that providing coconut water affects the growth of the number of leaves on robusta coffee seedlings. Coconut water given to experimental plants contains calcium (Ca) at 200.00 mg/L, cytokinin in zeatin at 14.97 mg/L, and zeatin at 14.97 mg/L. The element Ca is used to stimulate leaf growth. Based on the analysis of coconut water used in this study, it contains cytokinin, which affects the formation of buds that will develop into leaves. (Amsyahputra et al., 2016). The combination of planting media consisting of topsoil and cocopeat showed better results in the growth of the number of leaves. It is suspected that the combination of topsoil and cocopeat can provide sufficient nutrients for the growth of the number of leaves.

**Leaf Area.** The results of leaf area measurements tested by F at the 95% confidence level showed that treatment C (100% cocopeat) and treatment F (100% cocopeat + 200 mL coconut water) gave the same effect better than other treatments on the leaf area of robusta coffee seedlings. Treatments that used the same planting media showed results that were not significantly different. Treatments using cocopeat planting media showed the highest results. They were

significantly different from treatments using other planting media, such as treatment C (cocopeat 100%) and treatment F (cocopeat + coconut water 200 mL) significantly different from treatment A (topsoil 100%), treatment B (cocopeat 50% and topsoil 50%), treatment D (topsoil 100% + coconut water 200 mL), and treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) (Table 6).

It is suspected that the cocopeat planting media used contains nutrients carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and Mg, which play a role in the formation of chlorophyll and are useful in the photosynthesis process. Photosynthate will be metabolized into various organic compounds that play a role in cell formation, increasing leaf area. The N and Mg play a role in the photosynthesis process; if the photosynthesis process increases, it will produce carbohydrates and other compounds used by plants for growth, one of which is the growth of plant leaf area (Andri et al., 2017). Cocopeat can provide nutrients such as magnesium (Mg), potassium (K<sub>2</sub>O), and phosphorus (P) needed by plants. Cocopeat also contains zinc (Zn), which increases root growth and leaf expansion.

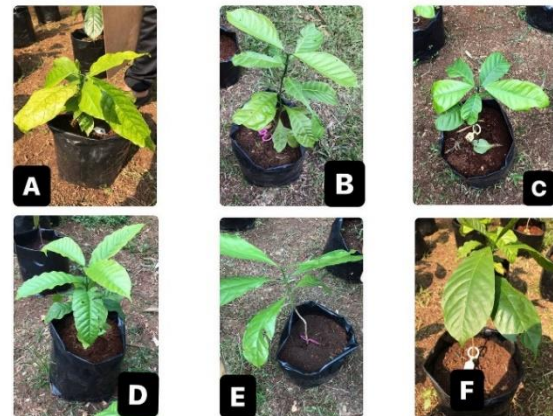


**Plant height.** The mean of plant height tested by F at the 95% confidence level showed that treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) and treatment B (cocopeat 50% and topsoil 50%) were treatments that gave a better effect than the other treatments. This shows that the treatment that uses a combination of planting media between topsoil and cocopeat is a treatment that gives a better effect on plant height parameters. This is also in line with research by Singchal (2019), which showed that the composition of planting media consisting of 50% soil and 50% cocopeat can increase the growth of coffee plant height (Table 7 and Fig 1)

Cocopeat can improve soil chemicals and physical properties by increasing the availability of macro and micronutrients and improving soil structure, water storage, and air exchange (soil aeration). Ramadhan (2017) showed that using 25% and 50% cocopeat combined with soil in the growing media of marine senton seedlings is the best composition for plant height growth. Table 7 shows that treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) is a treatment that gives a better effect than the other treatments. The provision of coconut water showed better results. The auxin and cytokinin content in coconut water plays an important role in cell division and helps the formation of buds and stem elongation. Auxin will spur cells to divide rapidly and develop into buds and stems (Pamungkas et al., 2009).

**Stem Diameter.** The results of stem diameter measurements tested by F at the 95% confidence level showed that treatment B (cocopeat 50% and topsoil 50%) was a treatment that gave a better effect than the other treatments but was not significantly different

from treatment E (cocopeat 50% and topsoil 50% + coconut water 200 mL) and F (cocopeat + coconut water 200 mL) (Table 8). This is in line with Singchal (2019), who states that the composition of planting media, 50% soil and 50% cocopeat, increases Arabica coffee plants' stem diameter growth.



**Figure 1. Morphological comparison of robusta coffee plant height as the effect of the combination of planting media and coconut water**

The availability of K element in the planting media influences stem diameter growth. The K element plays a role in accelerating the growth of meristematic tissues, especially in the stem of the plant, as well as strengthening the stem so that it does not easily fall over. According to Leiwakabessy (1998), the K element plays a very important role in increasing the diameter of the plant stem, especially in its network that connects the roots and leaves. Lack of K element can inhibit the process of stem circumference enlargement. The K element in the cocopeat planting media contains 3.92% K (Table 3).

**Table 7. Effect of the Combination of Planting Media and Coconut Water on the Plant Height of Robusta Coffee Seedlings at 4 - 12 WAP.**

Treatment	Plant height (cm)				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
A = control (topsoil 100%)	22.80 a	23.50 a	24.29 a	25.08 a	26.00 a
B = cocopeat 50% + topsoil 50%	35.29 c	36.50 c	37.75 c	39.81 c	41.18 c
C = cocopeat 100%	24.67 ab	25.29 ab	26.09 ab	26.67 ab	26.88 a
D = top soil 100% + coconut water 200 mL	22.45 a	23.63 a	25.92 a	28.63 ab	30.44 ab
E = cocopeat 50% + topsoil 50% + coconut water 200 mL	37.54 c	38.92 c	39.96 c	40.63 c	41.59 c
F = cocopeat 100% + coconut water 200 mL	28.00 b	30.21 b	31.33 b	32.33 b	33.31 b

Note : WAP = Week after planting. Mean followed by different letters within the similar column was significantly different based on Duncan multiple range test at the 5% level.

**Table 8. Effect of the Combination of Planting Media and Coconut Water on the Stem Diameter of Robusta Coffee Seedlings at 4 - 12WAP.**

Treatmet	Diameter of stem				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
A = control (topsoil 100%)	2.50 a	2.69 a	3.04 a	3.36 a	3.59 a
B = cocopeat 50% + topsoil 50%	4.67 c	4.89 c	5.24 c	5.74 b	7.03 d
C = cocopeat 100%	3.31 ab	3.54 ab	3.74 ab	3.98 a	4.23 ab
D = top soil 100% + coconut wat 200 mL	3.00 a	3.23 a	3.64 a	3.89 a	4.37 abc
E = cocopeat 50% + topsoil 50% + coconut water 200 mL	4.37 bc	4.77 bc	5.07 c	5.45 b	5.93 cd
F = cocopeat 100% + coconut water 200 mL	4.35 bc	4.64 bc	4.88 bc	5.17 b	5.48 bcd

Note : WAP = Week after planting. Mean followed by different letters within the similar column was significantly different based on Duncan multiple range test at the 5% level.

Another element that affects the growth of stem diameter of robusta coffee seedlings is the availability of P nutrients in the planting media. Cocopeat contains 23.31% P (Table 3). These nutrients play a role in helping the formation of plant organs, with the formation of carbohydrates that run well, and the translocation of starch to the stem will be smoother so that it forms a good stem diameter. Leiwakabessy (1998) stated that K and P elements play a very important role in increasing the diameter of the plant stem, especially in its role as a network that connects the roots and leaves. The availability of nutrients in cocopeat planting media helps in the growth of stem diameter of robusta coffee seedlings. The larger the stem diameter of plant seedlings, the better the plant growth.

**Leaf Chlorophyll Index.** Based on the results of the F test at the 95% confidence level, treatment C (100% cocopeat) significantly affected the chlorophyll index of robusta coffee seedlings. Treatment C (100% cocopeat) is the treatment that gives the best effect on the

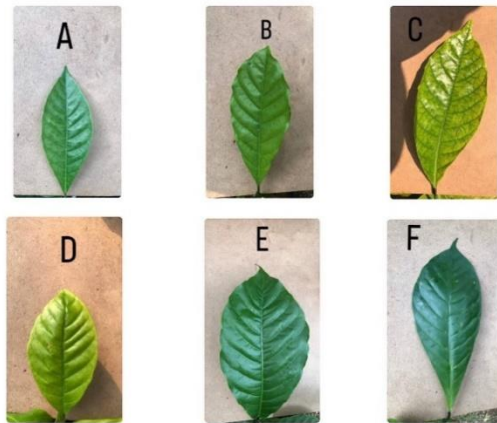
chlorophyll index parameter of the leaves. At the age of 4 weeks after planting, six weeks after planting, and 10 weeks after planting, the treatment using topsoil and the combination of planting media gave results that were not significantly different, while at the age of 8 weeks after planting and 12 weeks after planting, the treatment using the combination of planting media gave significantly different results (Table 9 and Fig.2).

The structure of the planting media produced by using cocopeat planting media spurs the process of cell division and elongation in plant organs, one of which is the growth of the leaf area. According to Musyarofah et al. (2007), leaf chlorophyll content is also influenced by a plant's morphological and anatomical structure. According to Rahhutami et al. (2021), chlorophyll, in the form of green leaf substance, occurs in the leaves with the help of sunlight and is called the process of photosynthesis. The magnesium (Mg) helps the formation of chlorophyll in the leaves, and this nutrient is containing in cocopeat planting media.

**Table 9. Effect of Combination of Planting Media and Coconut Water on Average Leaf Chlorophyll Index**

Treatment	Leaf chlorophyll index (CCI)				
	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP
A = control (topsoil 100%)	14.79 a	16.33 a	17.97 a	19.87 a	20.85 a
B = cocopeat 50% + topsoil 50%	20.10 bc	21.23 bc	22.15 bc	23.25 ab	23.73 ab
C = cocopeat 100%	33.31 d	34.73 d	36.10 e	37.65 c	38.31 d
D = top soil 100% + coconut wat 200 mL	16.96 ab	17.60 ab	18.85 ab	19.52 a	21.45 a
E = cocopeat 50% + topsoil 50% + coconut water 200 mL	23.60 c	24.83 c	26.17 d	27.42 b	30.73 c
F = cocopeat 100% + coconut water 200 mL	22.07 c	23.55 c	25.00 cd	26.10 b	28.24 bc

Note : WAP = Week after planting. Mean followed by different letters within the similar column was significantly different based on Duncan multiple range test at the 5% level.



**Figure 2. Leaf appearance comparison of robusta coffee as the effect of the combination of planting media and coconut water**

## Conclusion

Using cocopeat and coconut water could improve the growth of robusta coffee seedlings. The combination of topsoil 50% + cocopeat 50% had a better effect on the growth of the number of leaves, plant height, and stem diameter, while the composition of 100% cocopeat had a better effect on the growth of leaf area and chlorophyll index of robusta coffee seedlings.

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