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PREFACE

Kultivasi volume 24 (1) is the first edition of the year in 2025. Kultivasi is a journal that contains knowledge that focuses on showing crop production improvement in agronomy, pests and diseases, soil science, weeds, and plant breeding, while possibly benefiting others in cases related to crop development.

Starting this year, we have published 12 articles that have been reviewed by local and broad reviewers with a high credibility in their track record of writing in agriculture. We publish 3 times each year (April, August, and December). We can spread a lot of agricultural information to scientists and industry by this term.

Kultivasi journal is still open for contributions from other writers to increase the insight of readers and researchers in agriculture. Hopefully, in the future, we can continue to present agricultural sciences that make a real contribution to all stakeholders.

AUTHOR'S INSTRUCTIONS

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

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

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

Special Requirements

Review Articles:

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

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

Research Articles:

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

Title

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

Author's name

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

Abstract

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

Introduction

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

Materials and Method

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

Results and Discussions

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

Conclusion

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

Acknowledgment

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

Reference

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

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

CONTENTS

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Yudistira DH · Gunawan FA · Arif SA · Sato S

Role and key species of freshwater snails in enhancing plant growth performance of duckweeds (*Spirodela polyrhiza*) in rice paddy fields

Abstract. Freshwater snails are widely distributed in the rice field ecosystem. Freshwater snails' feeding and metabolic activities can potentially increase the complexity of freshwater ecosystems, particularly through the nutrient cycle. *Cipangopaludina chinensis*, *Physa acuta,* and *Semisulcospira libertina* are commonly found in Japan's paddy field ecosystems. *Spirodela polyrhiza* is expected to support biodiversity, improve soil fertility, and reduce greenhouse gas emissions in paddy field ecosystems, while providing food and shelter for the snails. In this study, we investigated the effects of these three snail species on the growth of *Spirodela polyrhiza*. The results showed that the presence of *P. acuta* or *C. chinensis* could promote the growth of *S. polyrhiza* compared with the presence of *S. libertina*, a mix, and the control. The relative growth rate for *S. polyrhiza* in the presence of *P. acuta*, *C. chinensis* + *P. acuta,* and *C. chinesis* was 1.10 ± 0.39, 1.10 ± 0.39, and 1.06 ± 0.31 fronds/day, respectively. In the *S. libertina* treatment, the number of *S. polyrhiza* fronds decreased as the snails consumed the plants. The number of fronds on the last day of treatment was 1.10 ± 1.10 fronds with a relative growth rate of 0.004 ± 0.030 fronds/day, whereas for the combination of *C. chinensis* + *S. polyrhiza* it was 4.80 ± 3.32 and 8.70 ± 3.61 fronds, respectively, with relative growth rate 0.13 ± 0.05 and 0.275 ± 0.06 fronds/day, respectively. Interestingly, these interspecific interactions increased *S. polyrhiza* performance, as indicated by greater frond length, root length, and frond chlorophyll content. This study highlights that interspecific interactions create complexity in the paddy field ecosystem, providing good conditions for biodiversity and indirectly supporting rice production.

Keywords: Biodiversity · Duckweed · Ecosystem engineering · Natural farming · Nutrient cycling

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Introduction

Freshwater snails are known to graze on macrophytes in the aquatic ecosystem. This activity enhances ecosystem stability and promotes coexistence in shallow aquatic systems (Lv et al., 2022), while also releasing nutrients into the environment after snails consume macrophytes. The nutrients released by snails can be beneficial for macrophyte growth. According to Pinowska (2002), the average phosphorus and nitrogen release rate by *Lymnaea* (Galba) *turricula* were 24.20±11.70 µg PO_4-P and 48.90±29.60 µg NH_4-N g^{-1} snail FW d^{-1}. Studies have observed that the presence of grazing snails strongly reduced nitrogen uptake and biomass of algae (Koleszár et al., 2021). According to Koleszár et al. (2021), in the absence of grazing freshwater snails, the *Ceratophyllum*-epiphyton complex lowered nitrogen and phosphorus concentrations in the medium more rapidly and exhibited higher pH levels compared to when snails were present. The results of research conducted by Jo et al. (2020) showed that over six years, using freshwater snails increases soil nutrients such as available nitrogen (N) and available phosphorus (P) compared to soil without freshwater snails. Additionally, applying freshwater snails increased soil pH in sodic soils. Also, these freshwater snails can serve as a biocontrol agent for reducing sodium adsorption, improving soil physical properties, and increasing infiltration rate (Jo & Pak, 2019). This finding is important for the development of organic farming with freshwater snails. From this perspective, freshwater snails are not harmful to the ecosystem. However, only a few studies have addressed the importance of interactions between freshwater snails in the ecosystem. These interactions can be divided into interspecific or intraspecific. Such interactions increase ecosystem diversity and can enhance ecosystem services such as nutrient cycling and decomposition of organic matter (Wilson et al., 2008).

Interactions occur not only between freshwater snail species but also with aquatic plants. In the rice field ecosystem, aquatic plants can play both beneficial and detrimental roles, since some species may become invasive or act as weeds (Bablee et al., 2024). Studies have shown that the relationship between freshwater snails and aquatic plants can be mutualistic. *Radix swinhoei* can increase the growth of submerged macrophytes by consuming epiphytes from the plant surface. As a result, not only do the plants grow better, but the biomass of freshwater snails also increases (Zhi et al., 2020). In this study, three species of freshwater snails were selected to examine the interspecific interaction on the growth performance of *S. polyrhiza* as a model plant. Duckweed, *S. polyrhiza,* is widely distributed in various freshwater ecosystems, mainly in rice field, and has several important roles, such as reducing the greenhouse effect of subtropical paddy fields (Wang et al., 2015), increasing yield as green manure (Yao et al., 2017), and increasing biodiversity (Fahmi et al., 2021). Additionally, duckweed provides favorable conditions for freshwater snails as shelters or food resources (Nagai et al., 1979). Certain species of freshwater snails commonly found in rice fields, such as *Cipangopaludina chinensis* and *Semisulcospira libertina* are native to Japan, and *Physa acuta* is an invasive species. All three species are widely distributed throughout Japan.

Some researchers have noted that *C. chinensis* plays a positive role in paddy plants and exerts indirect effects on the abundance of organisms in the terrestrial ecosystem (Dewi et al., 2017). Additionally, *C. chinensis* can affect the abundance of terrestrial organisms and may influence microbial communities directly or indirectly (Olden et al., 2013). In organic farming, the use of freshwater snails like *C. chinensis*, can play a significant role in enhancing nutrient cycling (Ernest et al., 2024; Kurniawan et al., 2018) and fostering ecosystem balance (Dewi et al., 2017; Kurniawan et al., 2018). Their ability to interact with aquatic plants, decompose organic matter, and regulate nutrient availability makes them valuable contributors to sustainable agricultural practices (Panteleit et al., 2018). For example, the grazing activity of these snails can help control unwanted aquatic weeds while simultaneously releasing nutrients that promote plant growth (Jong-Song et al., 2018). Additionally, nutrients excreted by these snails have been observed to increase rice plant performance (Kurniawan et al., 2018).

Based on these considerations, we hypothesize that *C. chinensis* is a key species in paddy field. The snail used in this experiment included *C. chinensis* because it is a native snail originating from various regions in Japanalong with *S. libertina*. In addition to these two snails, this experiment used *P. acuta* as an invasive freshwater snail in Japan. These three freshwater snails are not categorized as pests because they do not cause severe damage.

Materials and Methods

Preparation of Plants. Duckweeds were collected from a rice field in the Takasaka area of Tsuruoka, Yamagata Prefecture, Japan. After collection, the duckweeds were separated based on their species before being brought to the Applied Zoology Laboratory, Yamagata University. The experiment used *Spirodela polyrhiza* as the main duckweed species. Duckweeds were kept in 3-liter boxes filled with paddy soil and tap water without any additional nutrients. These boxes were set up in a rearing room within the laboratory, providing controlled environmental conditions for growth before they were used in experiments.

Collection of Animals. In this experiment, *C. chinensis*, *S. libertine*, and *P. acuta* were used. The first two are native snails originating from various regions in Japan, while *P. actuta* is an invasive freshwater snail in Japan. These three species were collected from the Yutagawa area, Tsuruoka, Yamagata Prefecture, Japan. Snails were maintained in 3.0 L boxes, and each species was put into a separate box. The boxes were filled with paddy field soil for snails to feed on. Snails were reared for one week before they were used in the experiment, allowing them to adapt to the laboratory environment.

Experimental Mesocosm Setup. The mesocosm experiment was conducted using a 535 mL plastic cup filled with tap water and 100 g of paddy soil. The paddy soil was collected from paddy fields at Yamagata University, Takasaka, Tsuruoka City, then dried and sieved to remove debris before use. The tap water was aged for 24 hours to reduce chromium levels before being added to the mesocosm.

After adding the water, a single frond of duckweed was introduced into each mesocosm and allowed to acclimate for 24 hours. Following this acclimation period, one individual snail from the designated species was introduced into each mesocosm. The setup was then placed in an incubator (Sanyo MIR 253) set to a temperature of 25 ± 1°C and humidity of 75%. The light cycle was controlled at 12 hours light : 12 hours dark (L:D 12:12 h) to simulate natural conditions. This experiment included four different treatments, with each treatment replicated ten times (Table 1).

Table 1. Design of treatment

Treatment (Code)	Description	Replication
C. chinensis (CC)	*C. chinensis* (width: 20.27±0.59cm) + *S. polyrhiza*	10
P. acuta (PA)	*P. acuta* (width: 1.02±0.02 cm) + *S. polyrhiza*	10
S. libertina (SL)	*S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
C. chinensis + *P. Acuta* (CCPA)	*C. chinensis* (width: 20.27±0.59cm) + *P. acuta* (width: 1.02±0.02 cm) + *S. polyrhiza*	10
S. Libertina + *C. chinensis* (SLCC)	*C. chinensis* (width: 20.27±0.59cm) + *S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
P. acuta + *S. Libertina* (PASL)	*P. acuta* (width: 1.02±0.02 cm) + *S. libertina* (width: 18.39±0.32cm) + *S. polyrhiza*	10
Control (C)	*S. polyrhiza*	10

Data Collection and Statistical Analysis. The number of duckweed fronds was counted every four days using a ruler. Every week, water pH data were recorded using a handheld Meyer (Marfied Eco pH Device). At the end of the treatment period, we measured biometric parameters of duckweeds, including root length, frond length, and fronds chlorophyll, using a handheld SPAD meter (Konica Minolta).

The relative growth rate (RGR) of *S. polyrhiza* was calculated using the formula:

$$RGR = (Xt-Xo)/dt$$

where Xt and Xo are the final and initial frond numbers, respectively, and dt is the duration of observation (days). To assess significant differences in duckweed growth, relative growth rate, pH, biometrics parameters, and freshwater snail survival, all data were tested for normality by the Shapiro-Wilk test and for homogeneity of variance by Bartlett's test. Biometric data were analysed using a one-way ANOVA followed by Tukey's HSD post-hoc test. Duckweed growth, RGR, pH, and snail survival data were analyzed with a Kruskal-Wallis test followed by the Kruskal-Nemenyi post-hoc test. All statistical analyses were performed in R (Version 4.0.3).

Results and Discussion

Effect of Freshwater Snail Interspecific Interaction on Plant Growth Performance. Over the past several decades, duckweed species have been studied for applications such as animal feed (Cruz-Velásquez et al., 2014; Mwale & Gwaze, 2013; Sońta et al., 2018), green manure for rice plants (Yao et al., 2017), and wastewater treatments (Chen et al., 2018; James, 2016). The growth of *S. polyrhiza* can be influenced by various factors such as nutrients in the aquatic environment, temperature, light intensity, and plant-animal interactions in the ecosystem (Fahmi et al., 2021; Jin et al., 2021; Strzałek & Kufel, 2021). One interesting finding is that the presence of freshwater snails and interspecific interaction between species can promote the growth of *S. polyrhiza*.

The results showed that *S. polyrhiza* grew well in the presence of *C. chinensis*, *P. acuta,* and their interaction ($X^2=39.06$, df=6, $P > 0.05$). In Figure 1, the *P. acuta* treatment had a growth rate of 1.28 fronds per day, followed by the interspecific combination *P. acuta* and *C. chinensis,* which showed a growth rate of 1.27, and then *C. chinensis* alone at 1.24 fronds per day. These three treatments were significantly different from the control ($X^2=39.063$, df=6, $P < 0.05$). These findings suggest that both freshwater snail species can enhance nutrient availability, consistent with several reports indicating that freshwater releases nutrients into the water, thereby promoting plant growth. Previous research showed that the presence of *B. aeruginosa* affected TN and TP content in the water (Mo et al., 2017). Some research also suggested *B. aeruginosa* could promote biomass and influence C, N, and P stoichiometry of submerged macrophytes like *Vallisneria natans* and *Hydrilla verticillata* (Li et al., 2019). According to Li et al. (2009), *Radix swinhoei* can release nutrients at a rate of approximately 0.66 μg NH_4^-N and 49.77 x 10-4μg PO_4^-Pmg−1 d−1 after consumption submerged plant. Adults of *Lymnaea turricula* can release 29.5μg NH_4^-N and 18.9μg $PO_4^-Pmg^{-1}\ d^{-1}$ even without consuming any plant material (Pinowska, 2002). Taken together, previous literature shows that nutrient release rates can differ among species and their activities. However, our study emphasizes the snail's effect on plant growth.

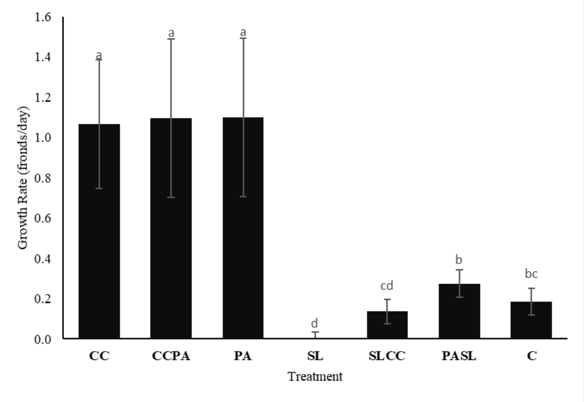

Figure 1. Relative growth rate of *S. polyrhiza* plant. CC= *C. chinensis*, CCPA= *C. chinensis* + *P. acuta*, PA= *P. acuta*, SL= *S. libertina*, SLCC= *S. libertina* + *C. chinensis*, PASL = *S. libertina* + *P. acuta*, C= control. Bars represent means ± standard errors. Different letters above bars indicate significant differences (P < 0.05) according to the Kruskal-Wallis test followed by the Kruskal-Nemenyi post-hoc test

The treatment with *S. libertina* showed the lowest mean frond count, reaching day 28 at 1.10 ± 1.10, which was significantly different from the control ($X^2=39.063$, df=6, $P < 0.05$). As shown in Table 2, there is a clear trend of decreasing fronds in *S. libertina* treatment compared to the mixture of *S. libertina* with *C. chinensis* or *P. acuta*. Interestingly, all treatments involving *P. acuta* and *C. chinensis* appeared to increase the number of fronds. The mean fronds when *S. libertina* was mixed with *P. acuta* were 8.70 ± 0.61, and when mixed with *C. chinensis* it reached 4.80 ± 3.32. These finding suggest that even a single individual snail can promote *S. polyrhiza* growth, and varying snail densities may not significantly affect its growth rate. This observation aligns with Li et al. (2019), who reported that different snail densities did not significantly influence the growth of *E. nuttallii* under low-nutrient conditions. According to these results, freshwater snail species could be a key factor in *S. polyrhiza* growth.

The growth of *S. polyrhiza has* been shown to benefit the performance of rice plants. When *S. polyrhiza* is present, it can reduce the density of weeds by 97% and increase yield by 23% (Wang et al., 2022). An abundance of duckweed during the early stage of rice plant growth can suppress weeds through competition for nutrients and light, as duckweed floats on the water surface (Yao et al., 2017). Moreover, high duckweed coverage decreased the pH value and water temperature while enhancing the SPAD value of rice plants (Jing et al., 2024). The rapid growth of duckweed can also be facilitated by the presence of snails (Ernest et al., 2024). Indeed, Ernest et al. (2024) found that the duckweed numbers increased more in the presence of snails compared to treatments without snails over a two-year experiment.

The treatment with *S. libertina* resulted in the lowest average frond count, with a mean of 1.10 ± 1.10 on day 28, which was significantly different from the control ($X^2 = 39.063$, df = 6, $P < 0.05$). The duckweed was consumed by *S. libertina* during the experiment. However, there is still a lack of information regarding the ability of *S. libertina* to consume duckweed. As shown in Table 2, there is a clear trend of decreasing frond numbers in the *S. libertina* treatment compared to treatments where *S. libertina* was mixed with *C. chinensis* and *P. acuta*. Interestingly, all treatments involving *P. acuta* and *C. chinensis* showed a potential increase in frond numbers. The mean frond counts for *S. libertina* mixed with *P. acuta* were 8.70 ± 0.61, while the mixture with *C. chinensis* resulted in a mean frond count of 4.80 ± 3.32.

Overall, our study indicates that the presence of certain freshwater snail species can be a key factor in promoting *S. polyrhiza* growth. These findings are consistent with Li et al. (2019), who found that different varying snail densities did not significantly affect *E. nuttallii* growth in low-nutrient environments, suggesting that snail-mediated effects are not strictly density dependent.

After 28 days of the growth experiment, we measured various plant biometric parameters. The results, presented in Table 3, show that *S. libertina* increased frond surface area compared to the control, though this difference was not statistically significant. Figure 2 shows that the number of fronds in the single-species *S. libertina* treatment was almost the same as control, while *C. chinensis* and *P. acuta* treatment showed a higher abundance of fronds. However, there were no significant effects on root length or chlorophyll content, which contrasts somewhat with the earlier observations of *S. libertina* reducing frond numbers. The combination of *C. chinensis* and *P. acuta* resulted in the longest fronds, with an average length of 0.60 ± 0.04 cm, nearly double that of the control. In terms of root length and frond chlorophyll content, *P. acuta* showed the highest average, followed closely by *C. chinensis*. The excretion of nitrogen and phosphorus by snails is known to enhance plant biomass (Edgar et al., 2022). Due to the rapid growth of the plants, intraspecific competition increased, leading to longer root growth (Jin et al., 2021). In this experiment, the combination of *C. chinensis* and *P. acuta* also promoted rapid biomass accumulation, likely due to phosphorus contributions, as noted by Yang et al. (2020). Furthermore, Jong-Song et al. (2018) found that saline rice fields supplemented with freshwater snails and half the usual fertilizer application produced yields comparable to those achieved with conventional chemical fertilizers. These findings suggest that freshwater snails can enhance plant growth even in challenging environments.

Table 2. Effect of freshwater snail presence on *S. polyrhiza* fronds growth

Treatment	Number of Fronds (fronds/day)						
	4	8	12	16	20	24	28
CC	1.70±0.21 a	2.70±0.67 ab	4.80±1.27 ab	7.90±2.62 a	16.00±5.73 a	22.00±7.40 a	30.90±5.41 a
CCPA	1.60±0.22 ab	2.10±0.38 ab	3.40±0.67 ab	6.30±1.81 a	14.50±5.21 a	20.60±6.35 a	31.70±5.50 a
PA	1.50±0.27 abcd	2.70±0.50 ab	4.90±1.20 ab	7.30±2.15 a	13.60±3.67 a	19.40±3.86 a	31.80±5.42 a
SL	0.80±0.20 d	0.80±0.29 c	0.30±0.30 d	0.30±0.30 c	0.20±0.20 d	0.60±0.60 d	1.10±1.10 d
SLCC	1.00±0.26 bcd	1.10±0.55 c	1.30±0.60 cd	1.70±0.70 bc	2.60±1.71 cd	3.00±2.05 cd	4.80±3.32 cd
SLPA	1.40±0.27 abc	3.70±0.63 a	5.00±0.87 a	6.40±1.28 a	7.00±1.82 ab	7.30±2.25 b	8.70±0.61 b
C	1.00±0.00 cd	1.50±0.17 bc	2.20±0.50 bc	2.60±1.28 b	2.80±0.29 bc	4.40±0.48 bc	6.20±0.89 bc

Note: Values are shown as means (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences according to the Kruskal-Wallis test (P < 0.05), followed by the Kruskal-Nemenyi post-hoc test.

Effect of Interspecific Interaction on Water pH. According to the data, the range of pH values in treatments with freshwater snail ranged between 5.32 and 5.82 (Table 5). This range seems not to have affected the growth of *S. polyrhiza*. However, interactions among *P. acuta* and *S. libertina* produced the lowest average pH value, whereas the presence of *C. chinensis* alone or in combination was associated with higher pH.

The observed pH conditions did not affect *S. polyrhiza* growth, as this species can grow in a wide pH range (3.0-10.0) (Mclay, 1976). Other researchers have noted that pH 6-8 does not influence duckweed growth but can increase the total ammonia (Caicedo et al., 2000). The average water pH may also influence freshwater snails. Chiu et al. (2002) found that *C. chinensis* survives in water pH ranging from 4.0 to 9.0, but its shell size is affected by pH. Cretini and Galloway (2024) reported that lower water pH conditions reduce shell size. Under acidic conditions, freshwater snails showed minimal shell length growth and increased shell erosion. Water pH condition is influencing the availability of calcium, which is crucial for shell formation. Lower water pH conditions can reduce calcium uptake, further inhibiting shell growth and strength (Glass & Darby, 2009; Grosell & Brix, 2009).

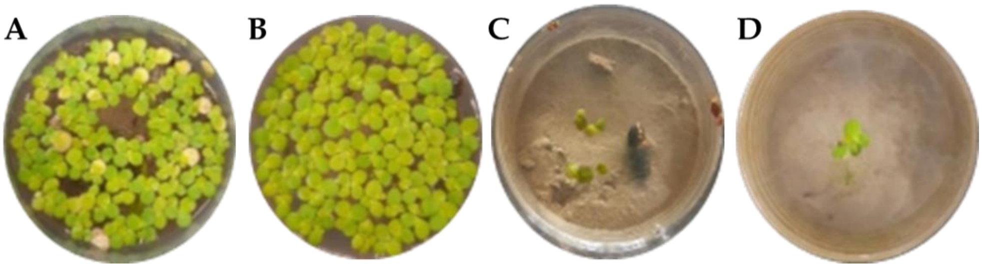

Figure 2. Effect of different freshwater snail species on the growth of *S. polyrhiza*. (A) *C. Chinensis*, (B) *P. Acuta*, (C) *S. Libertina*, and (D) Control

Table 3. Effect of freshwater snail presence on the final plant biomass of *S. polyrhiza*

Treatment	Biomass		
	Fronds Length (cm)	Root Length (cm)	Chlorophyll
CC	0.51±0.02 b	2.58±0.09 b	24.34±0.91 bc
CCPA	0.60±0.04 a	2.34±0.09 b	27.08±1.39 a
PA	0.46±0.02 bc	3.52±0.08 a	27.44±1.28 a
SL	0.42±0.02 bc	1.07±0.24 c	22.46±1.16 bc
SLCC	0.53±0.04 b	1.35±0.07 c	22.28±1.77 bc
SLPA	0.51±0.03 b	1.52±0.16 c	27.16±1.54 a
C	0.38±0.02 c	1.16±0.23 c	19.01±1.34 c

Note: Values are presented as mean (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences (P<0.05) according to Tukey's post-hoc test.

Table 4. Effects of Interspecific Interaction on Water pH

Treatment	Water pH (Week-)			
	1	2	3	4
CC	6.29±0.16 ab	5.95±0.08 ab	6.06±0.14 ab	5.82±0.09 ab
CCPA	6.13±0.12 ab	5.78±0.05 c	5.82±0.15 b	5.65±0.06 bc
PA	6.20±0.11 ab	5.57±0.07 d	5.50±0.05 c	5.48±0.05 cd
SL	6.41±0.18 a	6.01±0.06 a	6.04±0.08 ab	6.12±0.18 a
SLCC	6.24±0.10 b	5.93±0.04 ab	6.14±0.10 a	6.09±0.19 ab
SLPA	5.50±0.11 c	5.07±0.06 e	5.59±0.08 c	5.32±0.06 d
C	6.18±0.03 ab	5.87±0.02 bc	5.82±0.05b	5.45±0.06 d

Note: Values are presented as mean (± standard error). **CC** = *C. chinensis*, **CCPA** = *C. chinensis* + *P. acuta*, **PA** = *P. acuta*, **SL** = *S. libertina*, **SLCC** = *S. libertina* + *C. chinensis*, **SLPA** = *S. libertina* + *P. acuta*, **C** = control. Different letters within the same row indicate significant differences according to Kruskal-Wallis test (P < 0.05), followed by Kruskal-Nemenyi post-hoc test.

Freshwater Snail Survivorship and Interaction. Interspecific interactions play a key role in snail survival. The data show that the mortality of *S. libertina* alone in single-species treatments was six times higher than in the *C. chinensis* + *P. acuta* treatment. The percentage of mortality for *S. libertina* was 60%, but decreased when *P. acuta* was present. *Physa acuta* survived the experiment in all interspecific treatments. As shown in Table 2, *S. libertina* alone consumed *S. polyrhiza* fronds, leaving only 1.10 ± 1.10 fronds and exhibiting higher mortality, whereas *S. libertina* mixed with *C. chinensis* or *P. acuta* supported greater frond number, 4.80 ± 3.32 and 8.70 ± 0.61, respectively, and lower snail mortality. When *S. libertina* died under conditions where shell-attached algae grew rapidly in the mesocosm, it also inhibited the growth of *S. polyrhiza*.

Table 5. Freshwater snail mortality (%)

Species	Mortality Percentage (%)		
	C. chinensis	***P. acuta***	***S. libertina***
C. chinensis	10.00±10.00 bc	10.00±10.00 bc	20.00±13.33 bc
P. acuta	0.00±0.00 c	10.00±10.00 bc	0.00±0.00 c
S.libertina	0.00±0.00 c	30.00±15.28 b	60.00±16.33 a

Note: Values are presented as means (± standard error). Different letters within the the same row indicate significant differences (P<0.05) according to Tukey's post-hoc test

The increased mortality rate was likely caused by algae attached to the *S. libertina* shell and by the still water condition within mesocosms. The presence of other freshwater snails, such as *P. acuta*, could help reduce *S. libertina* mortality by half relative to the single-species treatment (Table 4), as it consumed algae attached to the *S. libertina* shell. Meanwhile, the presence of *C. chinensis* was important for *S. libertina* survival, as *C. chinensis* harbored shell-attached algae that *S. libertina* could feed on, reflecting *S. libertina*'s phytophagous diet as a food resource compared with *P. acuta,* which did not contain algae on the shell. *Semisulcospira libertina* in nature is a phytophagous diet (Antonio et al., 2010). Additionally, *C. chinensis* could help the growth of *S. libertina*'s shell by grazing on it. Algae rich in omega-6 fatty acids, commonly found in shell-attached biofilms, were a food source for *C. chinensis* after it fed on another snail's shell (Fujibayashi et al., 2016). This experiment revealed two key interactions among freshwater snail species and their effects on plant growth.

Conclusion

The presence of *P. acuta* resulted in the highest growth rate of *S. polyrhiza* (1.28 fronds/day), followed by the combination of *P. acuta* and *C. chinensis* (1.27 fronds/day). While *S. libertina* caused the lowest growth rate (1.1 fronds/day) due to high mortality and algae accumulation on shells, the presence of *C. chinensis* also positively influenced growth (1.24 fronds/day). The combination of *C. chinensis* and *P. acuta* achieved the best results for frond length (0.60 cm), while *P. acuta* alone had the highest root length (3.52 cm) and chlorophyll content (27.44). Treatments with *S. libertina* in combination with other snails improved survival and mitigated algae growth. Water pH was slightly affected but remained within a range suitable for *S. polyrhiza* growth.

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The interaction of nitrogen and potassium nutrients in increasing growth, yield, and quality of sweet corn products

Abstract. Fertilizer application is a strategy that can be used to increase sweet corn production and maintain environmental balance. This research aims (i) to determine the optimal dose of nitrogen and potassium fertilizers for sweet corn production; (ii) to study the agronomic and physiological responses of sweet corn plants at different nitrogen and potassium dose; and (iii) to determine the nitrogen (N) and potassium (K) nutrient uptake and efficiency by sweet corn at different fertilizer doses and their interactions on yield and yield quality. The research was conducted from March to November 2022 in the Faculty of Agriculture, Universitas Jenderal Soedirman. The experimental design used was a randomized block design. The treatments tried were N doses (0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_1), 200 kg ha^{-1} (N_2)) and K doses (0 kg ha^{-1} (K_0), 75 kg ha^{-1} (K_1), 150 kg ha^{-1} (K_3)). Each treatment was repeated three times. The observational data were analyzed using analysis of variance to determine the effect of treatment. If the results differed significantly, Duncan’s multiple range test (α=5%) was performed. Variables observed included growth traits, yield and yield components, physiological, biochemical, nutrient uptake and efficiency, and sweetness level. The experimental results showed that nitrogen dosage had a significant influence on growth traits, yield, and yield components, physiological, biochemical, nutrient uptake and efficiency, and sweetness level. The K dosage had a significant influence on N and K nutrient uptake. The interaction between a N dose of 100 kg ha^{-1} and a K dose of 150 kg ha^{-1} shows the best values for plant growth rate and potassium uptake.

Keywords: Efficiency · Nitrogen · Nutrient uptake · Potassium · Sweet corn

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Introduction

The demand for sweet corn (*Zea mays* saccharata) continues to increase, not merely due to its sensory appeal, such as sweeter taste and tender texture, but primarily because of its distinct end-use compared to field corn. Unlike regular corn which is mainly cultivated for animal feed, industrial raw materials, or grain processing, sweet corn is specifically grown for direct human consumption due to its high sugar content, lower starch level, and shorter harvest period (Jafarikouhini et al., 2020). This makes sweet corn more suitable for fresh markets, ready-to-eat products, and culinary uses, thereby driving its rising demand. The domestic productivity of sweet corn is still relatively low compared to other producing countries due to an unsuitable cultivation system. The average productivity of sweet corn in Indonesia is only 8.31 tons/ha, while the yield potential of sweet corn can reach 14-18 tons/ha (Supriyanta et al., 2023).

Market demand for sweet corn continues to increase, but major market opportunities cannot be fully exploited by Indonesian farmers and entrepreneurs due to various constraints (Godoy et al., 2014). It is believed that the growth and yield quality of sweet corn are influenced by environmental factors, particularly soil fertility. When managed properly, soil fertility can help make sweet corn production more cost-effective and labor-efficient, while ensuring consistently high yields.

Increasing sweet corn yield and quality will not result in high actual yields in the field without proper environmental and nutritional management. Fertilizer application is one of the main strategies to improve sweet corn productivity and maintain soil health (Hou et al., 2025). Nitrogen (N) is a macronutrient essential for vegetative growth, chlorophyll synthesis, and protein formation. However, the availability of N in the soil is naturally low, making fertilization necessary. Unfortunately, excessive or imbalanced application can reduce nutrient use efficiency and cause environmental harm (Barłóg et al., 2022; Kurniadie, 2002). The efficiency of N fertilization is reported to be only around 33% of the applied dose (Hameed et al., 2019).

In addition to nitrogen, potassium (K) is another crucial macronutrient that plays a key role in improving photosynthesis, strengthening cell walls, and regulating sugar accumulation in maize (Hafsi et al., 2014; Wu et al., 2024). Importantly, N and K have interactive effects in the soil-plant system (Sedri et al., 2022). Potassium helps balance excessive nitrogen uptake by preventing luxury consumption and promoting better nutrient distribution within the plant (Kang et al., 2015). A sufficient K supply enhances nitrogen use efficiency, while a K deficiency may worsen N-related imbalances, leading to poor plant structure and lower yields (Ye et al., 2021). Therefore, a balanced application of N and K is essential to optimize nutrient uptake, growth, and yield quality in sweet corn production systems.

Corn growth is affected by several environmental factors such as growing season, climatic conditions, water availability, and soil conditions, which can also affect sweet corn yield and quality. Soil moisture can significantly influence sugar content in sweet corn (Motazedian et al., 2019; Zarei et al., 2019). The presence of plant debris on the soil surface can also affect sweet corn plant growth and macronutrient mobilization (Mehta et al., 2020).

Sweet corn development in Indonesia is gaining traction among researchers, but adoption among farmers remains limited. To support optimal yield and quality, effective cultivation strategies—especially proper N and K management—are essential. Efficient nutrient uptake and tailored environmental practices are also critical components in improving productivity. This study aimed to: a) determine the optimal N and K fertilizer rates for sweet corn production; b) evaluate the agronomic and physiological responses of sweet corn to varying N and K levels; and c) assess the efficiency of N and K uptake and their potential interaction effects on yield and quality.

Materials and Methods

The research was conducted at the Experimental Farming area, Faculty of Agriculture, Universitas Jenderal Soedirman, Indonesia, situated at an altitude of 110 meters above sea level. The soil at the study site is classified as Inceptisol, with a dusty clay loam texture, a slightly acidic pH of 6.5, and a cation exchange capacity (CEC) of 19.96 cmol kg^{-1}. The soil has a C/N ratio of 12 and contains 2.47% organic carbon. In terms of nutrient availability, the soil

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contains 12 ppm nitrogen (N), 70 ppm phosphorus (P_2O_5), and 484 ppm potassium (K_2O), indicating moderate nitrogen content and high potassium availability. These baseline conditions provide critical context for evaluating the plant response to added N and K fertilizers in this study. The research was conducted from March to November 2022.

Materials used in this study were Bonanza sweet corn seed, aquadest, acetone, sulfuric acid, urea fertilizer, KCl fertilizer, chemicals for ANR analysis, nitrogen analysis, and potassium analysis, filter paper, tissue, and pesticides. Tools used include test tubes, graduated pipettes, measuring cups, erlenmeyer, beaker's glass, spectrophotometers, micrometers, microscopes, Kjedahl, flame photometers, SPAD chlorophyll meters, polybags, ovens, analytical scales, seed counters, scales, analytical scales, seed counters, porcelain cups, materials, and stationery.

The design used in this study was a factorial randomized block design. The factors tested were N and K fertilizer doses. The N fertilizer factor consisted of 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_1), and 200 kg ha^{-1} (N_2). The K fertilizer factor consisted of 0 kg ha^{-1} (K_0), 75 kg ha^{-1} (K_1), and 150 kg ha^{-1} (K_3). Nine combination treatments were repeated three times to obtain 27 experimental units. The plants were maintained with optimal irrigation (field capacity) and phosphate fertilization at a dose of 150 kg ha^{-1} in the form of SP-36 fertilizer.

The observed variables consist of growth variables (plant height (2, 4, 6, 8 weeks after planting/WAP), leaf area (3, 5, 7 WAP), leaf dry weight (75 days after planting/DAP), stomatal opening width (40 DAP), stomatal density (40 DAP), root length (3, 5, 7 WAP), shoot/root ratio (3, 5, 7 WAP) and plant dry weight (75 DAP)), yield variables and yield components (cob length, cob diameter, cob weight with husk and cob weight without husk), physiological characteristics (3, 5, 7 WAP), biochemical characteristics (7 WAP), nutrient uptake and nutrient use efficiency (7 WAP), yield and yield quality (harvest time).

1. Physiological characteristics consisting net assimilation rate (NAR) (Rajput, 2017) and relative growth rate (RGR).

   $$NAR = \frac{(W2-W1)(\ln(L2)-\ln(L1))}{(t2-t1)(L2-L1)}\ g\ cm^{-2}\ week^{-1}$$

   Where: W2 and W1 are the dry weight of the plants at the first and second destruction, L1 and L2 are the areas of the rice plants at the first and second destruction, t1 and t2 are the first and second destruction times of the plants.

   RGR was calculated using the method (Sridevi & Chellamuthu, 2015)

   $$RGR = \frac{W2-W1}{G\ (t2-t1)}\ g\ cm^{-2}\ week^{-1}$$

   Where: W1 and W2 are the dry weight of the plants at the destruction times t1 and t2. G is the land area occupied by plants.
2. Biochemical characteristics (content of chlorophyll a and b of the leaves using the colorimetric method with a spectrophotometer, as well as analysis of nitrate reductase (ANR) was determined with the Yoshida et al. (1976) method:

   Chlorophyll content was reported in Yoshida et al. (1976) measured using the Holden method (1965) by extraction with the organic solvent acetone. Rice leaf samples were collected at eight weeks after planting, with each sample weighed precisely at 0.1 g. The leaf samples were ground with a mortar and pestle, and 10 ml of 80% acetone was added. The leaf extract was filtered with Whatman No. 1 filter paper, and the solution was collected in a test tube. The absorbance of the chlorophyll extract solution was measured with a spectrophotometer at λ 645 nm and 663 nm. The chlorophyll content of the leaves was calculated using the following formula:

   Chlorophyll a = 0.0127 (OD 663) – 0.00269 (OD 645)
   Chlorophyll b = 0.0229 (OD 645) – 0.00468 (OD 663)
   Total Chlorophyll = 0.0202 (OD 645)– 0.00802(OD 663)

   Nitrate reductase (ANR) activity was reported in Yoshida et al. (1976) . Rice plant leaves were removed, cleaned with blotting paper and cut into small pieces. A total of 300 mg of leaf pieces was placed in a dark test tube, 5 ml of phosphate buffer pH 7.5 was added, and the leaves were soaked for 24 hours. After 24 hours, the buffer was discarded and replaced with 4.9 ml of fresh phosphate buffer, then 0.1 ml of 5 M NaNO3 solution was added as a substrate for nitrate reductase enzyme and incubated for 3 hours. The test tube was filled with 0.2 ml of 1% sulfanilamide solution and 0.2 ml of 0.02% naphthyl ethylene diamine solution in 3N HCl. A total of 0.1 ml of the solution was

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placed in a test tube filled with nitrite dye and allowed to wait for 10 to 15 minutes until it turned pink. 9.5 ml of distilled water was added to the solution. The solution in the test tube was shaken and transferred to a cuvette. Their absorbance was then measured at a wavelength of 540 nm using a spectrophotometer. ANR is expressed as the number of moles of nitrite formed per gram of fresh weight of the sample per hour.

$ANR = AsA0x1000Bx1Txx5001000\ \mu mol\ NO_2^-\ jam^{-1}$

Where: As: absorbance value of sample solution, Ao: standard absorbance value (0,0106 or 0,0142), B: Fresh weight of sample leaves (= 300 mg), T: incubation time.

3. Nitrogen analysis in plant tissue according to the Kjeldahl method (Yoshida et al., 1976). The nitrogen content in the tissue is then used to determine the nitrogen uptake of the sweet corn plants using the following formula:

   Nitrogen uptake = %N in plant tissue x dry weight

   The N use efficiency was measured using the method of Good et al. (2004), where the efficiency of agronomist was calculated using the following formula:

   $NUE\ (g\ g^{-1}) = Shoot\ weight/N$

   Where: NUE: Nitrogen Use Efficiency, N: % N in plant tissue.
4. Measurement of sweetness/sugar content with a digital refractometer.

The data obtained were analyzed using ANOVA, and if they differed significantly, they were further analyzed with the Duncan's multiple range test with a confidence level of 95%. A regression analysis was performed to obtain the optimal dose.

Results and Discussion

Observations on sweet corn plants included various parameters encompassing growth characteristics, physiology, biochemistry, yield and yield quality, and nutrient uptake and efficiency. Based on the analysis of variance in Table 1, nitrogen fertilizer application significantly influenced the growth characteristics of sweet corn. The dose of 200 kg ha^{-1} showed the most pronounced effect, particularly on plant height, leaf area, plant dry weight, and stomatal width. These findings are consistent with previous studies showing that nitrogen doses of 100 and 200 kg ha^{-1} result in optimal plant height (Zangani et al., 2021), leaf expansion, and stomatal development. The improved growth is attributed to nitrogen's role in promoting cell division and enlargement, especially in the apical meristems (Asfaw, 2022), as well as its essential function in chlorophyll and protein synthesis (Zayed et al., 2023).

However, the analysis did not reveal any significant interaction effects between nitrogen and potassium on all observed growth parameters. This absence of interaction might be due to the already sufficient availability of potassium in the soil (484 ppm K_2O), as indicated in the site's physicochemical characteristics. Under such high baseline potassium levels, additional K fertilization may not further enhance growth, thereby minimizing its interactive effect with nitrogen (Pandey, 2024). Moreover, potassium's primary physiological role tends to be more pronounced in later developmental stages such as carbohydrate translocation and stress resilience, rather than early vegetative growth, which is dominantly driven by nitrogen availability (Sustr et al., 2019).

Table 1. Effect of Nitrogen dan Potassium doses on the sweet corn growth characteristics

Treatment	Plant Heigh (cm)	Leaf Area (cm^2)	Shoot-Root Ratio	Plant Dry Weight (g)	Stomatal Width Opening (µm)	Stomatal Density (unit mm^{-2})
Nitrogen dose						
0 kg ha^{-1}	66.77 b	102.05 b	3.34 c	29.00 c	0.25 b	0.12 a
100 kg ha^{-1}	83.80 a	155.58 a	7.05 b	89.78 b	0.33 a	0.11 ab
200 kg ha^{-1}	85.58 a	162.33 a	8.86 a	108.45 a	0.34 a	0.10 b
Potassium dose						
0 kg ha^{-1}	76.42 b	142.51 a	6.49 a	76.26 a	0.32 a	0.11 a
75 kg ha^{-1}	78.36 ab	139.65 a	6.48 a	79.65 a	0.32 a	0.11 a
150 kg ha^{-1}	82.38 a	137.80 a	6.39 a	71.32 a	0.28 a	0.11 a

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

Statistically, the dose of 200 kg ha^{-1} nitrogen fertilizer showed higher values than the control value compared to the control and the dose of 100 kg ha^{-1} in terms of the parameters of shoot-root ratio and plant dry weight. Nitrogen utilization in plants can result in faster vegetative growth, elongated stems, larger leaves, and greener leaf color (Gardner et al., 1991). Higher dry weight indicates a more efficient and productive photosynthesis process, tissue cells develop larger and faster, and plant growth is better (Sembada et al., 2024). Table 1 shows that growth is manifested by an increase in size, reflects an increase in protoplasm, and is characterized by an increase in the dry weight of the plant (Utomo et al., 2016). The major components of plant dry matter are biomass, which consists of cell wall polysaccharides and lignin, as well as cytoplasmic components such as proteins, lipids, amino acids, and organic acids (Shivakumar et al., 2024).

Table 1 also shows that the parameters stomatal aperture width and stomatal density can be used to express the amount of leaf area that performs the photosynthesis process (Q. Yin et al., 2020). Stomatal closure is a plant response to limited water availability, minimizing water loss but also restricting CO_2 uptake, thereby reducing photosynthesis and inhibiting seedling growth. The amount of transpiration is also determined by the width of the stomatal aperture, which is caused by differences in turgor pressure in the guard cells. Increased stomatal resistance under stress indicates the efficiency of water conservation in the species (Widodo et al., 2016).

The results of the study also showed that the provision of potassium fertilizers had an effect on the variables of plant height, but had no effect on leaf area, shoot-root ratio, plant dry weight, stomatal width, and stomatal density. The provision of potassium fertilizer is not directly visible because the potassium element acts as an enzyme activator and can open and close stomata in the metabolism of plants, thus increasing photosynthesis and shifting photosynthesis from the leaves, which will later be used for the actively growing part, namely the apical meristem (Xu et al., 2020). K is a nutrient that is very sensitive to leaching, especially in tropical areas with high rainfall, which is why potassium in the soil is often considered a limiting factor (Bender et al., 2013). Potassium is taken up by plants in very large quantities and sometimes exceeds nitrogen, especially in tuber crops, but potassium availability is limited (Jan & Hussan, 2022).

The application of nitrogen fertilizer influenced several physiological and biochemical characteristics of sweet corn plants, as shown in Table 2. Although the nitrogen dose of 200 kg ha^{-1} numerically showed the highest values for relative growth rate (RGR), chlorophyll b content, and nitrate reductase activity (ANR), statistical analysis indicated no significant difference compared to the 100 kg ha^{-1} dose for RGR and chlorophyll-a content. Similarly, net assimilation rate (NAR) also did not differ significantly between the 100 and 200 kg ha^{-1} nitrogen treatments. However, both 100 and 200 kg ha^{-1} nitrogen doses significantly improved plant physiological and biochemical parameters compared to the control (0 kg ha^{-1}), particularly in terms of nitrate reductase activity and chlorophyll b content. Chlorophyll is a biochemical component and a key ingredient in the photosynthesis process, as well as a component of a plant's health indicator. Its interaction with water, temperature, nutrient availability, CO2, and sunlight can affect the rate of photosynthesis (Muñoz-Ortuño et al., 2017; Olivera Viciedo et al., 2021). Providing nitrogen at the right dose not only promotes the growth of maize plant weight, but also affects the increase in biomass productivity through photosynthesis and net assimilation (Pérez-Álvarez et al., 2024). This increase can also be caused by stopping the transfer of assimilates to male flowers. Since male flowers are absent, the assimilates are only directed to the producing parts that need them, namely the seeds (Kumar et al., 2023). Providing synergistic nitrogen will certainly also increase the seed yield and quality of maize seeds, and the interaction between leaf cuttings and male flower cuttings can also affect the distribution of assimilates between reproductive and vegetative organs (Heidari, 2013).

Nitrogen is a very important nutrient for plant growth (Leghari et al., 2016), as it functions as a part of amino acids, proteins, and chlorophyll pigment components that are important in the photosynthesis process (Fathi, 2022). When plants are supplied with the N element, they have a content of green leaf substance, which is very important for the process of photosynthesis (Bassi et al., 2018). Providing this element can also accelerate growth and increase plant height, which is influenced by the availability of nutrients such as nitrogen, phosphorus, and potassium (Razaq et al., 2017; Xu et al., 2020). Maize plants require high N content of nutrients, where N nutrients can be taken up by maize plants by 55-60%, P by 20%, and K by 50-70%. Lack of availability inhibits the growth and production of maize plants (Syafruddin et al., 2021).

Table 2 shows that potassium fertilization significantly affected the Net Assimilation Rate (NAR), but had no significant effect on Relative Growth Rate (RGR), chlorophyll a and b content, and nitrate reductase activity. Interestingly, among all the physiological and biochemical parameters, only RGR showed a statistically significant interaction between nitrogen and potassium applications (Table 3). The highest RGR value was observed in the 200 kg N ha^{-1} + 0 kg K ha^{-1} treatment (1.22 g dm^{-2} week^{-1}); however, this value was not significantly different from several other combinations, including 200 kg N ha^{-1} + 75 kg K ha^{-1} and 200 kg N ha^{-1} + 150 kg K ha^{-1}, which all shared the same statistical grouping. This indicates that while interaction exists, it does not show a clear superiority of one N–K combination over another within the tested levels. For other physiological variables, no significant interaction between N and K was found, suggesting that nitrogen plays a more dominant role in influencing sweet corn physiological traits, while potassium's effect might be more nuanced or masked under certain environmental conditions. Although potassium is crucial for photosynthetic efficiency and enzymatic activities (Hasanuzzaman et al., 2018; Xu et al., 2020), its independent effect may be limited under sufficient nitrogen conditions and specific environmental settings (Jalilian & Delkhoshi, 2014).

Table 3 shows that a significant interaction was observed between nitrogen and potassium doses on the relative growth rate (RGR). The highest RGR (1.07 g g^{-1} week^{-1}) was achieved under the combined application of 200 kg ha^{-1} nitrogen and 150 kg ha^{-1} potassium. This suggests that optimal N-K synergy enhances biomass accumulation, with nitrogen supporting vegetative growth and potassium facilitating physiological efficiency. Nitrogen fertilizer helps to increase the growth rate of plant tissue, for example, by stimulating the growth of young leaves (Rutkowski & Łysiak, 2023; Singh et al., 2014). The more leaves a plant produces, the more chlorophyll is absorbed by the leaves, which increases the yield of maize plants(S. Huang et al., 2017). Nitrogen is one of the nutrients that can directly and indirectly affect plant growth (Anas et al., 2020; Jaiswal et al., 2021) and plays a role in increasing the energy of plant cells by increasing the ion exchange capacity (Bloom, 2015). Potassium has a balancing effect on excess nitrogen in plants (Li et al., 2022). The requirement of potassium in maize plants varies depending on the needs of ongoing processes (Oosterhuis et al., 2014), such as photosynthesis and CO2 fixation, photosynthetic transfer to different users, and its relationship with water in plants (Yang et al., 2021).

Table 2. Effect of Nitrogen and Potassium doses on the sweet corn physiology and biochemical characteristics

Treatment	Net Assimilation Rate (g dm^{-2} week^{-1})	Relative Growth Rate (g dm^{-2} week^{-1})	Chlorophyll a Content (mg g^{-1})	Chlorophyll b Content (mg g^{-1})	Analysis of Nitrate Reductase (μmol NO$_2$ g^{-1} h^{-1})
Nitrogen dose					
0 kg ha^{-1}	2.16 b	0.41 b	0.017 b	0.006 c	2.881 c
100 kg ha^{-1}a	7.95 a	0.53 ab	0.026 a	0.012 b	5.121 b
200 kg ha^{-1}	7.85 a	0.56 a	0.028 a	0.016 a	6.537 a
Potassium dose					
0 kg ha^{-1}	6.25 ab	0.55 a	0.024 a	0.012 a	6.116 a
75 kg ha^{-1}	4.96 b	0.44 a	0.023 a	0.011 a	3.612 a
150 kg ha^{-1}	6.74 a	0.51 a	0.024 a	0.011 a	4.810 a

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

Table 3. Interaction between Nitrogen dan Potassium doses on the sweet corn relative growth rate

	Potassium		
Nitrogen	0 kg ha^{-1}	75 kg ha^{-1}	150 kg ha^{-1}
0 kg ha^{-1}	0.53 Bc	0.81 Ab	0.58 Bb
100 kg ha^{-1}a	0.90 Ab	0.91 Aab	1.07 Aa
200 kg ha^{-1}	1.22 Aa	1.06 Aa	1.07 Aa

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

Table 4. Effect of Nitrogen dan Potassium doses on the sweet corn yield and yield quality

Treatment	Cob Length (cm)	Cob Diameter (mm)	Cob Weight with Husk (g)	Cob Weight without Husk (g)	Sweetness Level (°brix)
Nitrogen dose					
0 kg/ha	17.81 b	37.50 c	111.39 c	75.25 c	9.00 b
100 kg/ha	**25.70 a**	51.86 b	296.99 b	195.95 b	14.00 a
200 kg/ha	26.41 a	**57.47 a**	**370.89 a**	**256.97 a**	14.89 a
Potassium dose					
0 kg/ha	23.37 a	48.51 a	242.38 a	170.85 a	12.44 a
75 kg/ha	22.56 a	48.50 a	264.06 a	171.75 a	12.33 a
150 kg/ha	24.00 a	49.81 a	272.84 a	185.57 a	13.11 a

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

Table 5. Effect of Nitrogen dan Potassium doses on the nutrient uptake and nutrient use efficiency

Treatment	Nitrogen uptake (%)	Potassium uptake (%)	Nitrogen use efficiency (%)	Potassium use efficiency (%)	Nitrogen agronomic efficiency (%)	Potassium agronomic efficiency (%)
Nitrogen dose						
0 kg/ha	10.38 c	28.45 c	24.77 b	9.47 b	-	-
100 kg/ha	78.81 b	184.04 b	62.91 a	26.10 a	85.53 a	-
200 kg/ha	116.62 a	241.57 a	62.10 a	29.95 a	55.87 b	-
Potassium dose						
0 kg/ha	65.42 b	141.97 b	50.84 a	23.17 a	-	-
75 kg/ha	63.75 b	143.58 b	54.72 a	23.46 a	-	17.34 a
150 kg/ha	76.64 a	168.51 a	44.27 a	19.78 a	-	12.95 a

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

The application of nitrogen fertilizer affected the yield and yield quality of sweet corn plants, as shown in Table 4. A nitrogen dose of 200 kg ha^{-1} significantly increased cob diameter, cob weight with husk, and cob weight without husk compared to the other treatments. Although cob length and sweetness level appeared higher in plants treated with 100 kg ha^{-1} nitrogen, statistical analysis showed no significant difference between the 100 and 200 kg ha^{-1} nitrogen doses for these two variables. Nitrogen fertilization improved cob weight significantly, with increases of 33.87%, 56.02%, and 77.97% observed at doses of 50, 100, and 200 kg ha^{-1}, respectively, demonstrating the positive role of nitrogen in enhancing yield components (Afrida et al., 2024). Proper fertilization during the maize growth period increases maize yield if applied properly (gradually) to prevent leaching or evaporation (N. Wang et al., 2023). Nitrogen is important for the production and storage of carbohydrates. Therefore, plants that produce large amounts of carbohydrates have a high nitrogen requirement (Gardner et al., 1991). One of the functions of nitrogen is to improve the quality of fruits during the reproductive period (Novizan, 2002). Nutrients taken up by plants are used to form proteins, carbohydrates and fats (Švarc et al., 2022), which are then stored in the seeds and increase the weight of the cobs (Govender et al., 2008).

Table 4 shows that there was no significant interaction between nitrogen and potassium treatments on any of the yield and yield quality parameters, including cob length, cob diameter, cob weight with husk, cob weight without husk, and sweetness level. Additionally, the application of potassium fertilizer alone did not result in any significant improvements in these traits. One possible explanation is the limited availability of potassium in the soil due to its chemical form. Only 1–2% of total soil potassium exists in an exchangeable and plant-available form, while the majority—approximately 18–20%—is fixed in mineral structures and not readily accessible to plants (Buckman & Brady, 1982). This slow-release nature of potassium may have reduced its effectiveness within the crop cycle, especially if the timing of availability did not align with the critical growth phases of sweet corn.

The availability of nutrients required by plants can be ensured by adding suitable nitrogen fertilizers to accelerate the uptake of nutrients (Plett et al., 2020). This can certainly lead to the optimum production of plants. Nitrogen is the most important supporting element for the growth process, development, and determining the quality of plant yields (Hameed et al., 2019). Plants require large amounts of nitrogen to grow. Nitrogen is also the most important macronutrient that makes up proteins and is the main constituent of protoplasm, chloroplasts, and enzymes (Kumar et al., 2023). The role of nitrogen is related to photosynthetic activity and is therefore required for metabolism and respiration (Noor et al., 2023).

The application of nitrogen fertilizer significantly influenced nutrient uptake and nutrient use efficiency in sweet corn (Table 5). Nitrogen doses of 100 kg ha^{-1} and 200 kg ha^{-1} both led to significantly higher nitrogen uptake, potassium uptake, and nitrogen agronomic efficiency compared to the control. Among these, the 200 kg ha^{-1} dose showed the highest values. However, for nitrogen use efficiency and potassium use efficiency, there was no statistically significant difference between the 100 kg ha^{-1} and 200 kg ha^{-1} treatments, indicating that increasing nitrogen beyond 100 kg ha^{-1} did not further improve the efficiency of nutrient utilization. This suggests that while higher nitrogen input boosts nutrient uptake, it does not necessarily translate into more efficient nutrient use (Table 5). Plants absorb about 33% of the total nitrogen supplied, the rest is lost through chemical and biological processes (Fageria & Baligar, 2005). To achieve optimal results, a correct and appropriate dosage is required when applying nitrogen to sweet corn plants, as increasing the nitrogen dosage to sweet corn does not necessarily result in an increase in yield under the same cropping system (Wang et al., 2023).

Optimal nitrogen supply can support optimal plant growth and production. Sufficient nitrogen supply is characterized by high photosynthetic rates, good vegetative growth, and dark green plant color (M. Huang et al., 2019; Munawar, 2011). Increasing nitrogen use efficiency in sweet corn shows that providing nitrogen at the right dose can increase its use efficiency, so that it can support plant growth, physiological, and production processes (Tamagno et al., 2024). Nitrogen uptake by plants increases with the addition of nitrogen fertilizer, and increased nitrogen uptake is associated with increased nitrogen availability in the soil (Govindasamy et al., 2023; Liu et al., 2017). Nitrogen functions as a component of amino acids, proteins, chlorophyll, nucleic acids, and coenzymes (Munawar, 2011; X. M. Yin et al., 2014). Higher N doses lead to stronger plant growth (Qiao et al., 2013).

Table 5 indicates that there was no significant interaction between nitrogen and potassium doses on nutrient uptake and efficiency variables, including nitrogen use efficiency (NUE), potassium use efficiency (KUE), and nitrogen agronomic efficiency (NAE). Table 5 also showed that the provision of potassium fertilizers had an effect on the variables of nitrogen uptake and potassium uptake. Plants with a potassium application rate of 150 kg ha^{-1} showed the best potassium application rate for the variables nitrogen uptake and potassium uptake. This is probably because the total potassium content in the soil is high enough for plants to take up potassium well. The amount of potassium that plants take up is determined by several factors, including the potassium concentration in the soil (Sardans & Peñuelas, 2021; Torabian et al., 2021). Potassium is an element that moves in plant cells, plant tissues, both xylem and phloem. Potassium in the cytoplasm and chloroplasts is needed to neutralize the solution to a pH of 7-8. This pH environment provides an optimal reaction process for almost all plant enzymes. When plants are deficient in potassium, many processes such as carbohydrate accumulation, reduced starch content, and accumulation of nitrogen compounds in plants do not function properly (Hasanuzzaman et al., 2018).

Potassium uptake is strongly influenced by the presence of macronutrients in the soil. These nutrients can help improve the photosynthesis process, increase water use efficiency, form stronger stems and strengthen roots to keep plants healthy, and increase plant resistance to disease (Medrano et al., 2015). N nutrients act as building blocks for proteins, chlorophyll, amino acids, and many other organic compounds (Gardner et al., 1991). When plants have an excess of nitrogen, potassium plays a role in restoring the balance (Xu et al., 2024).

Meanwhile, there is no effect on nitrogen use efficiency, potassium use efficiency, and potassium agronomic efficiency with the provision of potassium fertilizers. Each element has a solubility within a certain pH range. If the pH is too high, the solubility of the element is reduced, which can reduce the plant’s ability to absorb it or make elements such as potassium inaccessible to plants. As a result, the roots show symptoms of potassium deficiency, such as stunted growth and reduced production (Geng et al., 2023).

The nitrogen dose of 100 kg ha-1 showed the best effect on the nitrogen agronomic efficiency, compared to the dose of 200 kg ha-1, but did not show an effect on the potassium agronomic efficiency (Table 5). Agronomic efficiency (AE) is the efficiency of applied nutrients in increasing grain or biomass yield. It is calculated as the increase in yield per unit of nutrient applied (Brouder & Volenec, 2017; Vaneeckhaute et al., 2014). Agronomic efficiency is useful in assessing how much production increase is achieved by the specific amount of fertilizer added (Davies et al., 2020). Agronomic N-use efficiency is the basis for economic and environmental efficiency, and an effective agro-ecosystem management practice, improving nutrient use efficiency, is a crucial challenge for a more sustainable production of horticultural, industrial, and cereal crops (Montemurro & Diacono, 2016).

Conclusion

Based on the results of this study, a nitrogen fertilizer dose of 200 kg ha^{-1} significantly increased cob diameter, cob weight (with and without husk), nitrogen uptake, potassium uptake, and nitrogen agronomic efficiency, indicating its effectiveness in enhancing sweet corn yield components and nutrient absorption. However, no significant difference was observed between the 200 kg ha^{-1} and 100 kg ha^{-1} doses for cob length, sweetness level, nitrogen use efficiency, and potassium use efficiency. Therefore, while 200 kg ha^{-1} supports optimal performance in several key parameters, a dose of 100 kg ha^{-1} may be more efficient for certain physiological traits. Regarding the applied potassium dose, it has not been shown which dose is best for the growth, physiology, and yield of sweet corn. Moreover, the 200 kg ha^{-1} dose results in higher nitrogen and potassium absorption values compared to other doses. On the other hand, applying a nitrogen fertilizer dose of 100 kg ha^{-1} gives better results in terms of nitrogen efficiency, potassium efficiency, and nitrogen agronomic efficiency. A potassium fertilizer dose of up to 150 kg ha^{-1} can increase the uptake of nitrogen and potassium. There is no interaction between nitrogen and potassium doses and nutrient uptake or nutrient use efficiency, and agronomic efficiency.

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Effect of meta-topolin and kinetin at various concentrations on shoot multiplication in white turmeric (*Curcuma zedoaria* Rosc.)

Abstract. White turmeric is a medicinal plant widely used to treat various diseases. The limited availability of white turmeric seedlings is influenced by the scarcity of quality seeds due to the long dormancy of the rhizomes and the high incidence of pathogen attacks. Tissue culture techniques are one alternative to address the problem of white turmeric seedling availability. This study aims to determine the best concentrations of meta-topolin and kinetin for in vitro shoot multiplication of white turmeric. The research was conducted from January to June 2022 at the tissue culture laboratory, Faculty of Agriculture, Universitas Padjadjaran. This study used a completely randomized design consisting of 7 treatments: control (0 mg L^{-1}), meta-topolin (1 mg L^{-1}; 2 mg L^{-1}; 3 mg L^{-1}), kinetin (1 mg L^{-1}; 2 mg L^{-1}; 3 mg L^{-1}). Analysis of variance (ANOVA) and Duncan's multiple range test were used to analyze the statistical effects of meta-topolin and kinetin application. Applying 1 mg L^{-1} meta-topolin yielded the best results for shoot number, indicating that 1 mg L^{-1} meta-topolin is a potential cytokinin for the propagation of *Curcuma zedoaria* Rosc.

Keywords: *Curcuma zedoaria* Rosc. · Kinetin · Meta-Topolin

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Introduction

White turmeric (*Curcuma zedoaria* Rosc.) is a rhizomatous medicinal plant belonging to the Zingiberaceae family, widely used to treat various diseases (Hashiguchi et al., 2022). Turmeric holds significant value as a biopharmaceutical commodity in Indonesia and is primarily used in the formulation of herbal medicines. These species have long served as a cornerstone of traditional Indonesian treatments due to their rich therapeutic properties and diverse active compounds (Rahmat et al., 2021). The rhizome of white turmeric plays a crucial role in treating diseases such as diarrhea, gout, hypertension, and also has properties as an anticancer, antibacterial, antidiabetic, and neuroprotective agent (Hashiguchi et al., 2022). The compounds found in the rhizome of white turmeric include flavonoids, flavonols, stigmasterol, chalcones, and β-sitosterol (Hashiguchi et al., 2022).

In Indonesia, turmeric farming is widespread across nearly all regions, covering a total harvested area of 7,481,396 hectares. East Java stands out as the region with the largest turmeric output (117,108,216 tons), followed by Central Java (25,747,866 tons) and West Java (4,183,745 tons) (Central Bureau of Statistics, 2020), indicating fluctuating productivity.

The challenge of reliance on imported medicinal raw materials in Indonesia's pharmaceutical production stems from the limited availability of local simplicia. This issue is particularly evident with white turmeric, where the limited availability of quality seedlings — only accessible once a year during the rainy or dry season — hampers its cultivation. While white turmeric can be propagated vegetatively through rhizomes, Indonesian farmers have not widely adopted this method. As a result, the price remains high, reaching 90,000 IDR/kg in 2021. Additionally, the long dormancy period of 7-8 months for rhizomes, necessary for full maturity (Krishna et al., 2020), further complicates the availability of seedlings and impacts production.

The limited availability of white turmeric seedlings is partly due to pathogen attacks, including yellow mold (*Aspergillus flavus*) (Krishnakumar et al., 2021), rhizome rot (*Pseudomonas fluorescens*) (Prabhukarthikeyan et al., 2017), and bacterial wilt (*Ralstonia solanacearum*) (Seow-neng et al., 2017). One solution to this problem is tissue culture. Tissue culture is a propagation method that isolates plants aseptically to produce fast-growing, disease-free plants (Espinosa-Leal et al., 2018). This technique is based on the theory of cellular totipotency, which holds that each cell has the ability to grow into a complete plant, genetically identical to the parent (Su, et al., 2021).

The success of plant propagation and development is highly dependent on the growing medium and plant growth regulators (PGRs), one of which is cytokinin. Cytokinins are a group of plant hormones that play a role in cell division, such as promoting lateral bud growth, stimulating stomatal formation, inhibiting leaf senescence, encouraging leaf expansion, and promoting chloroplast development (Wybouw & De Rybel, 2019). Cytokinins that can be used to stimulate shoot formation in white turmeric include meta-topolin and kinetin.

According to the study by Waman et al. (2021), applying meta-topolin at a concentration of 1 mg L^{-1} efficiently promotes shoot growth in *Curcuma mangga* plants. The activity of meta-topolin is effective in delaying leaf senescence and significantly reduces the rapid decomposition of chlorophyll in leaf segments, thereby maintaining higher total chlorophyll content during senescence (Işlek, 2021). Additionally, the application of meta-topolin has been associated with increased protein retention, further contributing to delayed senescence. In comparison with benzyladenine (BA), meta-topolin demonstrates several advantages, including a reduced tendency to induce vitrification and better support for overall plant health and rooting efficiency.

The application of kinetin at concentrations of 2 mg L^{-1} and 1 mg L^{-1} NAA is optimal for shoot regeneration in *Kaempferia angustifolia* Roscoe (Haque & Ghosh, 2018). However, the application of cytokinin at very low concentrations is unable to enhance cell division activity for shoot growth. In contrast, excessively high concentrations fail to promote growth as they inhibit the shoot formation process. This study evaluates the efficacy of meta-topolin and kinetin at different concentrations for in vitro shoot induction of white turmeric. The goal is to identify the most effective cytokinin treatment and concentration for shoot regeneration, develop an efficient micropropagation protocol, and increase seedling availability to support sustainable cultivation of this valuable medicinal plant.

Materials and Methods

Time and Place. The experiment was conducted from January to June 2022. The experiment occurred in the Tissue Culture Laboratory of Seed Technology, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor.

Materials. The planting material used was white turmeric rhizome shoots obtained from the Biofarmaka Cultivation Conservation Unit (UKBB), Tropical Biofarmaka Study Center, Research and Community Service Institute, IPB University.

Medium Composition. The in vitro culture medium consisted of Murashige and Skoog (MS) medium, agar Gelzan, sucrose, aquades, and cytokinin-type PGR (meta-topolin and kinetin). Other materials used outside the medium preparation included 70% alcohol, 1 N NaOH, 1 N HCl, *n*-hexane, ethyl acetate, ethanol, methanol, acetone, quercetin solution, 10% $AlCl_3$, 1 M CH_3COONa, and 1 M CH_3COOK.

Methods

Experimental design. The method used was an experimental method with a Completely Randomized Design (CRD), consisting of 7 treatments and 4 replications. The treatments included a control (0 mg L^{-1}), three concentrations of meta-topolin (1 mg L^{-1}, 2 mg L^{-1}, and 3 mg L^{-1}), and three concentrations of Kinetin (1 mg L^{-1}, 2 mg L^{-1}, and 3 mg L^{-1}). The PGR treatments were added during the preparation of the culture medium, by incorporating the specified concentrations of meta-topolin or kinetin into the Gelzan-based MS medium before pouring the medium into culture vessels.

Data analysis was performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. The experiment was carried out in the following steps: (1) sterilization of equipment, (2) preparation of media, (3) preparation of explants, (4) planting of explants, and (5) observations conducted at 12 weeks after planting (WAP), including measurements of shoot number, root number and length, number of leaves, plant height, and fresh weight.

Analysis of total chlorophyll content. The total chlorophyll content was measured after the explants were destroyed, following the method of Batubara et al. (2016). Five milligrams of white turmeric leaves were ground and mixed with 250 ml of n-hexane, then extracted using ethyl acetate as a solvent. The extract solution (100 mg L^{-1}) was dissolved in methanol:HCl:water (90:1:1) and transferred into a microtube. The supernatant was collected and placed into a cuvette, then measured at wavelengths of 663 nm and 645 nm using a UV-Visible spectrophotometer. Total chlorophyll content was calculated using the following formula (Arshad et al., 2023):

$$\frac{total\ chlorophyll\ (mg)}{leaf\ weight\ (g)} = [20.2\ (A645) + 8.20\ (A663)\ x\ \frac{Vv}{1000\ x\ W}$$

Where:
A : Absorbance
V : Volume of chlorophyll extract
W: Weight of leaf

Analysis of total flavonoid content (TFC). The total flavonoid content was measured after the plantlets were extracted, following the method of Naznin et al. (2019). The leaves were ground and extracted using ethanol as a solvent up to the boundary mark. The extract solution was transferred into seven 10 ml volumetric flasks (0 mg g^{-1}, 1.6 mg g^{-1}, 3.2 mg g^{-1}, 4.8 mg g^{-1}, 6.4 mg g^{-1}, 8 mg g^{-1}, 9.6 mg g^{-1}). To each flask, 3 mL of methanol and 0.2 mL of $AlCl_3$ were added and allowed to react with a minute. The solution was then diluted with distilled water and incubated for 40 minutes, and the absorbance was measured at a wavelength of 415 nm using a UV-Visible spectrophotometer. The total flavonoid content in the sample was expressed in QE mg/100 g using the following formula V T Nguyen et al (2020)::

TFC (mg QE/100 g) = $\frac{C.V}{M}$
Where:
C : Concentration of quercetin (mL^{-1})
V : Volume of extract (ml)
M: Mass of extract (g)

Results and Discussion

Number of Shoots. The success of shoot multiplication is characterized by the number of shoots that develop. An increase in the number of shoots correlates with a higher multiplication rate of the plant. A high shoot multiplication rate positively influences the production of seedlings. According to the analysis of variance, the application of 1 mg L^{-1} meta-topolin yielded the highest average number of shoots compared to the other treatments (Table 1). This finding is consistent with the research by Waman et al. (2021), which demonstrated that applying single meta-topolin (without auxin combination) at a concentration of 1 mg L^{-1} effectively promotes shoot growth in *Curcuma mangga* plants.

According to Gantait, S., & Mitra, M. (2021), meta-topolin (mT) has been shown to not only support the efficient induction of multiple shoots, similar to or better than BA, but also to minimize physiological disorders commonly associated with BA and TDZ, such as hyperhydricity and inhibited rooting. This contributes to improved shoot quality and overall success in the micropropagation of horticultural species. Meta-topolin is also considered more active in shoot multiplication than zeatin, Kinetin, and BA, making it a viable alternative cytokinin to replace BA in inducing shoot formation (Gantait, S., & Mitra, M., 2021).

Meta-topolin is a potent cytokinin effective in stimulating shoot organogenesis across various plant species. It activates cell division receptors (AHK3) with higher affinity than other cytokinins, promoting enhanced shoot formation (Gantait & Mitra, 2021). In cassava, a two-stage protocol with meta-topolin improved shoot regeneration by over 35% in cultivar TME 7 (Chauhan & Taylor, 2018). However, shoot induction by meta-topolin is concentration-dependent, with higher concentrations potentially reducing shoot numbers, such as at 2 mg L^{-1}. These results underscore meta-topolin's potential in promoting shoot organogenesis, though its effectiveness varies with concentration.

Table 1. Differences in shoots number in response to meta-topolin and kinetin application

Treatments		Number of Shoots
A	(Control)	0.86 a
B	(1 mg L^{-1} meta-topolin)	3.75 b
C	(2 mg L^{-1} meta-topolin)	1.19 a
D	(3 mg L^{-1} meta-topolin)	1.63 a
E	(1 mg L^{-1} kinetin)	1.19 a
F	(2 mg L^{-1} kinetin)	1.00 a
G	(3 mg L^{-1} kinetin)	1.50 a

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

According to Waman et al. (2021), applying excessively high concentrations of meta-topolin in explants can result in a loss of shoot quality and abnormal shoot development. Increasing cytokinin concentrations may reduce shoot elongation, thus hindering shoot multiplication. Table 1 shows that explants cultured in the control medium, which did not receive any cytokinin application, produced the lowest number of shoots. This is likely due to the insufficient endogenous cytokinin alone to stimulate shoot formation. When exogenous cytokinin was added, it induced the formation of shoots at twice the number compared to the control. The addition of exogenous plant growth regulators, such as cytokinins, can regulate apical dominance by stimulating the formation of axillary shoots. Therefore, when applied at high concentrations, cytokinins can induce shoot formation (Wybouw & De Rybel, 2019).

The number of roots. Roots are essential for the absorption of water and nutrients, and an increased root number enhances the plant's ability to efficiently fulfill its nutritional demands. In environments with abundant nutrients, root systems exhibit morphological adaptations, such as the growth of additional root hairs, which facilitate more effective nutrient uptake (Farhangi-Abriz, S., et al., 2023). These structural modifications in the root system are critical for optimizing nutrient absorption, enabling plants to thrive in diverse conditions. Table 2 shows that applying 3 mg L^{-1} Kinetin resulted in a higher average number of roots, although the difference was not statistically significant compared to the 2 mg L^{-1} Kinetin treatment.

The observed Root growth in explants is primarily driven by high endogenous auxin levels, which are essential for root development, including organogenesis and response to environmental stimuli (Cavallari et al., 2021). Auxin regulates the architecture of the root system, influencing both primary and lateral root formation. Additionally, the presence of cytokinins in explants boosts ethylene production, further promoting adventitious root formation. Wounding during explant preparation stimulates the synthesis of endogenous auxins and other growth regulators, which are critical for root development (Pasternak & Steinmacher, 2024). Together, endogenous auxins and external cytokinins play a vital role in root formation and successful in vitro plant regeneration.

Table 2. Differences in roots number and length in response to meta-topolin and kinetin application

	Treatments	Number of Roots	Length Roots (cm)
A	(Control)	3.65 a	5.38 a
B	(1 mg L^{-1} meta-topolin)	4.69 a	4.82 a
C	(2 mg L^{-1} meta-topolin)	4.94 a	4.91 a
D	(3 mg L^{-1} meta-topolin)	4.46 a	5.38 a
E	(1 mg L^{-1} kinetin)	4.92 a	6.81 b
F	(2 mg L^{-1} kinetin)	8.25 b	8.59 c
G	(3 mg L^{-1} kinetin)	9.44 b	7.71 bc

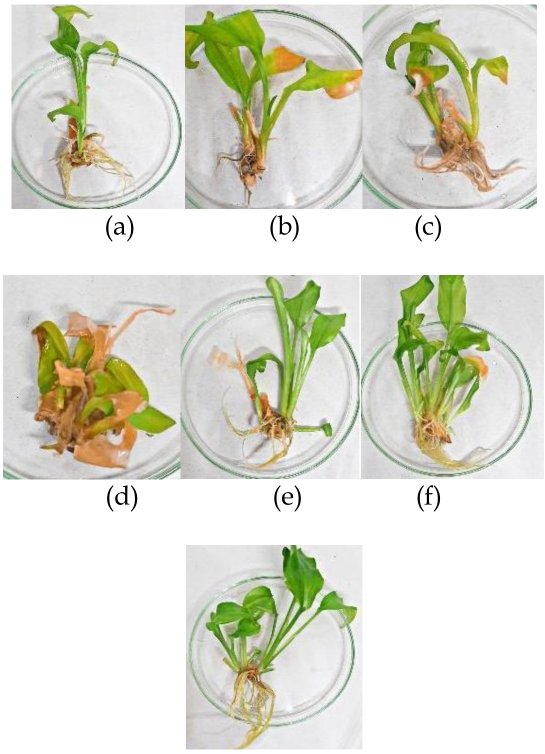
Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

The analysis of variance results show that the longest root length was observed in the 2 mg L^{-1} kinetin treatment. According to Yasin et al. (2018), kinetin is a type of cytokinin that is crucial in stimulating the apical meristem in cell division and elongation, thereby enhancing root elongation. Kinetin also increases the accumulation of photosynthates in the roots, leading to greater root elongation due to the balance of nutrients across the plant's tissues (Al-Zubaidi et al., 2020). However, in this study, increasing the kinetin concentration did not correspond to an increase in root length. This is likely because the endogenous auxin in the explants was already sufficient to induce root formation and elongation. Thus, exogenous cytokinin application was not required in high concentrations.

The control and meta-topolin treatments did not result in a statistically significant difference in root number or length, indicating that endogenous auxin levels were adequate to support root development. As highlighted by Alaguero-Cordovilla et al. (2021), wounding stimulates localized auxin biosynthesis, which plays a critical role in the initiation of adventitious roots. Root formation is largely determined by the internal concentration of auxin and its synergistic interaction with cytokinins.

The application of meta-topolin at all concentrations resulted in short roots, as observed in the explant appearance at 12 weeks after planting (Figure 1). This is consistent with the findings of Shekhawat et al. (2021) who reported that applying meta-topolin alone is less effective for root induction; however, its combination with auxin significantly improves root characteristics. Combining meta-topolin with auxin is suggested to be more effective for achieving optimal root induction and elongation (Gantait, S., & Mitra, M., 2021).

Number of Leaves. The leaves are an important plant organ as they are directly involved in light-capture processes such as photosynthesis. The number of leaves is a component that indicates plant growth; the more leaves present, the greater the light absorption by the leaves for photosynthesis. Based on the analysis of variance, all treatments did not show a significant difference in the number of leaves (Table 3).


(a) (b) (c)

(d) (e) (f)

(g)

Figure 1. Differences in plantlet morphology at12 weeks after culture: (a) control; (b) 1 mg L^{-1} meta-topolin; (c) 2 mg L^{-1} meta-topolin; (d) 3 mg L^{-1} meta-topolin; (e) 1 mg L^{-1} kinetin; (f) 2 mg L^{-1} kinetin; and (g) 3 mg L^{-1} kinetin

Table 3. Differences in leaves number in response to meta-topolin and kinetin application

	Treatments	Number of Leaves
A	Control)	2.65 a
B	1 mg L^{-1} meta-topolin)	4.94 a
C	2 mg L^{-1} meta-topolin)	3.88 a
D	3 mg L^{-1} meta-topolin)	4.82 a
E	1 mg L^{-1} kinetin)	4.21 a
F	2 mg L^{-1} kinetin)	4.25 a
G	3 mg L^{-1} kinetin)	5.19 a

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Table 3 shows that the number of leaves tended to increase with the application of kinetin, although the increase was not significant. This indicates that although an increase in shoots generally corresponds with an increase in the number of leaves, this is not universally applicable. Leaf development is governed by a combination of hormonal balance and genetic factors, which regulate the function of the shoot apical meristem and leaf initiation. The interaction of these factors can result in variations in leaf production that are independent of the number of shoots (Peng, et al., 2023). Consequently, the application of kinetin to explants did not yield a significant difference in the number of leaves, as there was no substantial variation in the number of shoots produced.

In the meta-Topolin treatment, vitrification symptoms were observed in the leaves of white turmeric with the application of meta-topolin (Figure 1). These symptoms increased with higher concentrations of meta-topolin applied. The application of cytokinins such as BA (benzyladenine) at excessively high concentrations can increase the number of leaves exhibiting vitrification (Gantait, S., & Mitra, M., 2021). Vitrification in plants, particularly in micropropagated species, is a physiological disorder marked by a decrease in stomatal density and an increase in stomatal size, which results in abnormal leaf development. This condition is primarily triggered by high humidity and nutrient-rich culture media that interfere with normal leaf morphology and physiological processes. One of the key features of vitrified leaves is the malfunctioning of stomata, which tend to remain open and unresponsive to environmental cues, thereby causing excessive water loss. This stomatal dysfunction is further exacerbated by structural abnormalities in the cell wall, such as hypolignification and reduced cellulose biosynthesis, which impair stomatal regulation and overall leaf function (Marques et al., 2021).

Plant Height. Plant height is an important component for assessing the response of explants to the treatments applied, as it reflects the growth rate after cultivation. Plant height affects the plant's ability to capture light and the leaf area index. Table 4 shows that the application of 3 mg L^{-1} kinetin resulted in a higher average plant height compared to the meta-topolin treatment. However, this difference was not statistically significant when compared to the control, 1 mg L^{-1} kinetin, and 2 mg L^{-1} kinetin treatments. This is consistent with the study by Gawande et al. (2020), which found that the application of kinetin in *Curcuma longa* significantly resulted in the highest plant height compared to other cytokinin treatments. Kinetin can enhance the activity of both apical and lateral meristems, thereby promoting vegetative growth such as plant height (Khandaker et al., 2018).

Table 4. Differences in plantlet height in response to meta-topolin and kinetin application

	Treatments	Plant Height (cm)
A	Control)	7.12 ab
B	1 mg L^{-1} meta-topolin)	6.11 a
C	2 mg L^{-1} meta-topolin)	6.06 a
D	3 mg L^{-1} meta-topolin)	6.13 a
E	1 mg L^{-1} kinetin)	7.10 ab
F	2 mg L^{-1} kinetin)	7.69 ab
G	3 mg L^{-1} kinetin)	8.15 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

The shortest plant height in Table 4 was observed in explants treated with meta-topolin. The height of the explants is influenced by the number of shoots formed. Based on the number of shoots produced, the explants treated with meta-topolin had a large number of shoots, but the plants were short. As the number of shoots increases, energy and resources are diverted from stem elongation to shoot formation, resulting in a reduction in overall plant height. Wang et al. (2018) suggest that this process is governed by the plant's hormonal balance, which determines whether growth is prioritized for shoot development or elongation, thereby influencing the overall growth dynamics.

Fresh weight. Fresh weight refers to the weight obtained from all parts of the plant, including the roots, stems, and leaves. Fresh weight is closely related to vegetative growth; the better the growth, the greater the fresh weight produced. Based on the analysis of variance, the treatment with 3 mg L^{-1} kinetin resulted in the highest fresh weight compared to the other treatments (Table 5).

This is consistent with the study by Chuengpanya et al. (2020), which found that the application of kinetin in *Hedychium longicornutum* plants resulted in the highest fresh weight compared to other cytokinins. Kinetin plays a role in enhancing the efficiency of photosynthesis, which aids in the accumulation of nutrients throughout the plant. As a result, fresh weight increases due to the balanced distribution of nutrients within the plant tissues (Al-Zubaidi et al., 2020).

Table 5. Differences in plantlet fresh weight in response to meta-topolin and kinetin application

Treatments		Fresh Weight (g)
A	Control)	1.83 a
B	1 mg L^{-1} meta-topolin)	2.68 a
C	2 mg L^{-1} meta-topolin)	1.98 a
D	3 mg L^{-1} meta-topolin)	1.89 a
E	1 mg L^{-1} kinetin)	1.81 a
F	2 mg L^{-1} kinetin)	2.59 a
G	3 mg L^{-1} kinetin)	3.85 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Table 5 shows that the lowest fresh weight of explants was observed in treatment E, where the explants were applied with 1 mg L^{-1} kinetin, resulting in an average fresh weight of 1.81 g. This may be because the exogenous cytokinin application at this concentration is insufficient to achieve maximal fresh weight in the explants. According to Li et al. (2018), an increase in fresh weight in plants is in line with the concentration of kinetin applied. The higher the kinetin concentration applied to the plant, the greater the fresh weight. Therefore, explants treated with 3 mg L^{-1} kinetin were the most effective in producing the highest fresh weight in white turmeric plants.

Total chlorophyll content. Chlorophyll is the green pigment found in chloroplasts, playing a crucial role in photosynthesis. The chlorophyll content in the leaves serves as an indicator of the plant's metabolic balance. According to the analysis of variance, the treatment with 2 mg L^{-1} kinetin resulted in the highest average total chlorophyll content, measuring 0.94 mg g^{-1} (Table 6). This is in line with the findings of Yaowachai et al. (2020), who reported that kinetin helps maintain chlorophyll stability in *Globba globulifera* plants. Li et al. (2018) state that kinetin can preserve chlorophyll stability and enhance antioxidant enzyme activity. The application of kinetin has been shown to improve the formation of photosynthates and increase chlorophyll content in plants (Gurnami et al., 2018).

Table 6. Differences in plantlet chlorophyll content in response to meta-topolin and kinetin application

Treatments		Total Chlorophyll Content (mg/g)
A	Control)	0.70 b
B	1 mg L^{-1} meta-topolin)	0.58 a
C	2 mg L^{-1} meta-topolin)	0.53 a
D	3 mg L^{-1} meta-topolin)	0.49 a
E	1 mg L^{-1} kinetin)	0.54 a
F	2 mg L^{-1} kinetin)	0.94 c
G	3 mg L^{-1} kinetin)	0.71 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Kinetin enhances photosynthetic pigments and chlorophyll content in leaves, increases CO_2 assimilation, boosts photosynthesis rates, and extends the period of active photosynthesis. This occurs because kinetin can promote stomatal conductance and increase the number of chloroplasts in leaves by stimulating cell growth and cytoplasmic activity, thereby increasing chlorophyll synthesis (Khandaker et al., 2018). An increase in kinetin concentration correlates with an increase in the length and width of chloroplasts in plants, meaning that higher kinetin concentrations result in higher chlorophyll content (Li et al., 2018).

Total flavonoid content (TFC). Flavonoids do not directly participate in the normal growth of plants; however, they play an active role in the survival of plants. Flavonoids are a group of polyphenolic compounds with biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, and anti-viral properties, and the ability to inhibit cancer development. Based on the variance analysis, the application of 2 mg L^{-1} kinetin, showed the highest average total flavonoid content of 1.21 mg g^{-1} (Table 7). This is in line with the findings of Gantait, S., & Mitra, M. (2021), who reported that kinetin can enhance secondary metabolite content, specifically promoting flavonoids (anthocyanins) in *Haplopappus gracilis* plants. According to Gurav et al. (2020), secondary metabolites are closely related to cell differentiation, meaning that the increase in total flavonoid content highly depends on the plant growth regulators applied.

Table 7. Differences in total flavonoid content in response to meta-topolin and kinetin application

Treatments		Total Flavonoids Content (mg g^{-1})
A	Control)	1.17 f
B	1 mg L^{-1} meta-topolin)	0.77 e
C	2 mg L^{-1} meta-topolin)	0.46 a
D	3 mg L^{-1} meta-topolin)	0.58 c
E	1 mg L^{-1} kinetin)	0.52 b
F	2 mg L^{-1} kinetin)	1.21 g
G	3 mg L^{-1} kinetin)	1.72 d

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Flavonoids are generally plentiful in older leaves and are a main defense mechanism for plants. Flavonoids absorb UV radiation, primarily accumulating in the epidermis of leaves, thus shielding underlying tissues from damage (Ferreyra et al., 2021). This is one of the reasons why the total flavonoid content was assessed in 12-week-old leaves. The high total flavonoid content is also influenced by the conditions in which the plant is grown. According to Yaowachai et al. (2020), in vitro conditions can enhance flavonoid and antioxidant activity in *Globba globulifera*. This occurs because the nutrient and hormone levels remain stable in in vitro conditions, which in turn increases the synthesis and gene expression of secondary metabolites. Table 7 shows that the application of kinetin at the highest concentration (3 mg L^{-1}) did not correlate with an increase in total flavonoid content. This may be due to the fact that the endogenous cytokinin content in the explants was already sufficient to enhance the total flavonoid content in the leaves, meaning that a higher concentration of cytokinin was not necessary.

Conclusion

This study demonstrates that the application of meta-topolin and kinetin at varying concentrations significantly affects the growth of white turmeric explants, influencing key parameters such as shoot and root development, plant height, root length, fresh weight, and the accumulation of total chlorophyll and flavonoids. Among the treatments, 1 mg L^{-1} meta-topolin was the most effective in promoting shoot proliferation, suggesting its potential as an optimal regulator for in vitro propagation. These findings provide valuable insights into the hormonal control of white turmeric explant growth and offer a foundation for enhancing propagation protocols, with important implications for both the sustainable cultivation and commercial production of this medicinal species.

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The effect of weed control using herbicide on soil bacteria, growth, and yield of sweet corn

Abstract. Weeds are managed by herbicides, but this can reduce the abundance of soil bacteria. This research aimed to determine the effect of active compounds of herbicides on weeds, the abundance of soil bacteria, growth and yield of sweet corn. The experiment was conducted from June to October 2023 in the fields and Laboratory of Agronomy and Horticulture, Universitas Jenderal Soedirman, Purwokerto. A randomized block design was used, consisting of eight treatments and four replications. The treatments included control (H0); weeding (H1); paraquat (H2); glyphosate (H3); paraquat, atrazine, mesotrione (H4); glyphosate, atrazine, mesotrione (H5); paraquat, atrazine, mesotrione, nicosulfuron (H6); and glyphosate, atrazine, mesotrione, nicosulfuron (H7). Results showed that glyphosate and paraquat, were applied before planting, could suppress weeds on sweet corn until 15 days after planting (DAP). Application of atrazine, mesotrione, and nicosulfuron at 21 DAP can increase the success of weed control observed up to 35 DAP of sweet corn. The application of glyphosate, atrazine, and mesotrione showed the highest values for growth variables (plant height, number, and leaf greenness index) and yield variables (fresh weight, diameter, and length of sweet corn cob). Herbicide decrease abundance of soil bacteria on sweet corn field, from $10.07x10^{-8}$ CFU/mL to $9.55x10^{-8}$ CFU/mL (total bacteria), $9.53x10^{-8}$ CFU/mL to $9.52x10^{-8}$ CFU/mL (phosphate solubilizing bacteria), $9.90x10^{-8}$ CFU/mL to $9.40x10^{-8}$ CFU/mL (Rhizobium) and $9.91x10^{-8}$ CFU/mL became $9.78x10^{-8}$ CFU/mL (nitrogen fixing bacteria). The total density of phosphate-solubilizing bacteria and nitrogen-fixing bacteria in the application of glyphosate, atrazine, and mesotrione (7.78 CFU/g and 9.52 CFU/g) was greater than control (8.77 CFU/g and 8.15 CFU/g).

Keywords: Herbicide · Soil bacteria · Sweet corn · Weeds

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Introduction

Corn is the second main food after rice, which is a source of carbohydrates and has high economic value. Sweet corn has a taste that is liked by people, which causes sweet corn's demand to increase (Sidahmed et al., 2024), but its productivity is still low (8.31 tons/ha) compared to the potential yield (14–18 tons/ha) (Sunari et al., 2022). Weeds are obstacles for sweet corn cultivation because they compete for resources like sunlight, water, and growing space (Asih et al., 2018). Weeds can decrease corn yields up to 10-15 percent, even up to 20-80 percent if not controlled (Radjabov et al., 2025). Herbicide application has been a standard agricultural practice to control weeds because it is effective and efficient in time, energy, and costs (Espig et al., 2022).

The effectiveness level of determined by the active ingredients contained. Farmers usually use herbicides containing the active ingredients glyphosate and paraquat. Glyphosate is a systemic and non-selective herbicide, so that not only the target weeds but also the main plants can die due to improper application (Martinez et al., 2018). Paraquat is a non-selective contact herbicide that can penetrate weed organs and react in them to produce hydrogen peroxide, which can damage cell membranes in all plant organs, showing the effect of burning plants (Chen et al., 2021).

Application of herbicides to corn fields is generally done before planting to make planting easier. Therefore, when corn enters the vegetative phase, weeds have started to grow and can disturb the corn. Kurniadie, et al., (2022) reported that up to the age of 28 DAP the ability of the herbicide paraquat decreased in its ability to control weeds. The usage of non-selective paraquat and glyphosate herbicides can be combined with selective post-emergence herbicides such as mesotrione, nicosulfuron, and atrazine to increase their effectiveness (Giraldeli et al., 2019).

Herbicide applications can also have negative impacts on the environment and living creatures (Bruggen et al., 2021). Herbicides affect the agroecosystem and the activity of non-target organisms such as soil microbes, which play a role in increasing the absorption of nitrogen, phosphorus, and potassium in situ, as well as the production of siderophores, which indirectly provide a source of nutrition for plants (Jeyaseelan et al., 2024). Herbicide residues can enter through plants to the root area or directly into the soil (Fuchs et al., 2023). The effect of herbicide application can be inhibiting, activating, or showing no effect on soil microorganisms (Bharathi et al., 2024). Glyphosate affects the number of microbes and the enzymatic activities in the rhizosphere (Lupwayi et al., 2022). Pose-Juan et al. (2017) reported that high-dose application of triasulfuron reduced the number of soil microbes. Tyagi et al. (2018) showed there were differences of soil bacteria population, fungi, and actinomycetes after 5 days of herbicide application compared to before herbicide application. Based on Xu et al. (2022), application of herbicides containing sterane could reduce the variety and density of soil bacteria in corn fields at 10 DAP, but they increase again by 60 DAP. Meanwhile, Fernandes et al. (2020) stated that atrazine could increase atzA and trzN genes in Brazilian Red Latosol soil, where these residues did not cause significant changes in the long-term structure of the bacterial community. Therefore, effect of herbicide on soil microbes still needs further research (Chen et al., 2021).

This research aims to determine the effect of active compounds herbicides, i.e., paraquat, glyphosate, atrazine, mesotrione, and nicosulfuron, in controlling weeds in corn plantations, and their effect on the growth and yield of sweet corn and the abundance of soil bacterial populations. It is hoped that this research will provide benefits for researchers, institutions, and the general public, especially farmers, to find out the types of herbicides that are safe for the abundance of soil bacteria but effective in controlling weeds in corn plantations.

Materials and Methods

Experimental Materials. This research used materials, including 'Exsotic' sweet corn seeds produced by PT. Agri Makmur Pertiwi, distilled water, acetone, urea fertilizer produced by PT. Petrokimia Gresik, KCl MerokeMOP® fertilizer, TSP-46 MerokeTSP® fertilizer, tissue, paraquat (Gramoxon), glyphosate (Roundup), atrazine-mesotrion (Gandewa), nicosulfuron (Neocron), nutrient Agar media, NFb media, yeast-monitol agar media, pikovskaya media, cotton, spirits, and pesticide Dangke 40WP. The tools used include laminar air flow, test tube (Pyrex), measuring cup (Pyrex), Erlenmeyer (Pyrex), beaker glass (Pyrex), SPAD (Konica Minolta), oven (Lab-Line Instruments), 16L sprayer, analytical balance (Paj1003), petri dishes, labels, envelope paper, and stationery.

Land Preparation. This research was conducted in the fields and Laboratory of Agronomy and Horticulture, Universitas Jenderal Soedirman Purwokerto at an altitude of approximately 74 masl with the Inceptisol soil classification. This research was conducted on Mei until October 2023. Land preparation using a no-tillage system. The land was plotted into 32 plots consisting of 8 treatments and 4 replications. Each plot measures 5 m x 5 m was marked with a treatment label and bounded with rope. After the land is prepared, sweet corn seeds are planted into holes 2-3 cm deep at a distance of 60 cm x 30 cm. The hole is covered with compost and watered to keep the soil moist.

Application of Herbicides. This research used randomized block design (RBD) consisting of one factor (weed control) with eight treatments replicated four times. The treatments are:

H0 = control (without weeding)
H1 = weeding
H2 = paraquat
H3 = gyphosate
H4 = paraquat, atrazine, mesotrione
H5 = glyphosate, atrazine, mesotrione
H6 = paraquat, atrazine, mesotrione, nicosulfuron
H7 = glyphosate, atrazine, mesotrione, nicosulfuron

Weeding was applied every 2 months. Paraquat and glyphosate were applied one week before planting. Atrazine, mesotrione, and nicosulfuron were applied at 21 DAP of corn. The herbicide application doses are 4 L/ha for glyphosate, 3 L/ha for paraquat, and 2 L/ha for atrazine+mesotrione and nicosulfuron.

Data Collection Procedures

The variables observed in this research included:

Weed Identification. Weed identification was carried out 3 times, i.e., before planting, 15 DAP, and 35 DAP of corn. Weed sampling before planting was carried out by taking weeds directly using a quadrat method measuring 0.5 x 0.5 m in experimental fields. Weed sampling points before planting were chosen randomly and carried out ten times. Weed sampling after planting was also carried out using the quadrat method in each experimental plot. The data analysis process was proposed to determine the type and dominance of weeds in the area. The dominant weed type is determined by looking for the Summed Dominance Ratio (SDR) value. The SDR value is obtained from calculating the relative density of a species, the relative dominance of a species, the relative frequency of a species, and the importance value index (Tsytsiura, 2020).

$$SDR = \frac{RD+RF+RD'}{3}$$

Growth and Yield of Sweet Corn. The growth and yield variables observed included sweet corn growth (number of leaves, leaves greenness index, and plant height) and sweet corn yield (length, weight, and diameter of corn cobs). Leaves greenness index measured by SPAD meter for the base, middle, and tip of the 3rd leaf from the topmost shoot. Cob weight was measured by weighing corn cobs with husks and without husks, the cobs length was measured from base to tip of the cobs both with husks and without husks, and the diameter of the corn was measured from the most bulging part of the cob using a caliper.

Population density of soil bacteria. To calculate the population density of soil bacteria, soil samples are first taken to isolate the bacteria, carried out before planting, and after harvest. Soil sampling before planting was chosen deliberately (purposive sampling) on the experimental land (figure 1), while soil sampling post-harvest was carried out on each treatment plot (figure 2). It was carried out using a diagonal system. The number of points was set 5 points with a distance of ± 32.5 m from the center point. Soil was taken at a depth of 0-20 cm, then the five samples were mixed (Liu et al., 2021).

10 grams of soil samples were put into Erlenmeyer flask containing 90 mL of distilled water, then shaken until homogeneous using a shaker. The 1 mL sample solution was placed in a test tube containing 9 mL of distilled water and diluted 10^{-8}. 1 mL of each dilution was taken and placed in a sterile petri dish, then the petri dish was poured with solid NFb media, YMA, Pikovskaya, and NA media using the pour plate method.

Calculation of the total bacteria, nitrogen fixing bacteria, phosphate solubilizing bacteria, and rhizobium uses the total plate count (TPC) method. The number of bacteria is expressed as colony farming units (CFU) (Martini et al., 2023). The following is the formula for calculating TPC:

$$\text{Bacteria Population} = n \frac{1}{\text{Dilution Factor}} \text{ CFU/g}$$

Information:
n : The number of colonies contained in the 10x dilution series tube
CFU/g : Colony forming unit/g

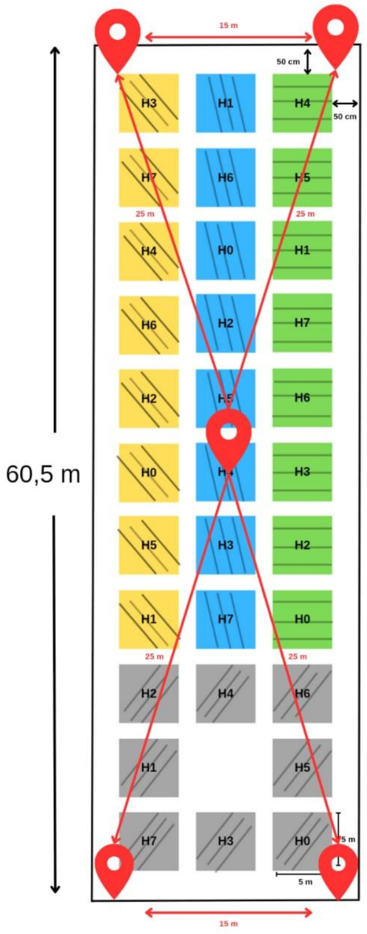

Figure 1. Soil sampling point before planting

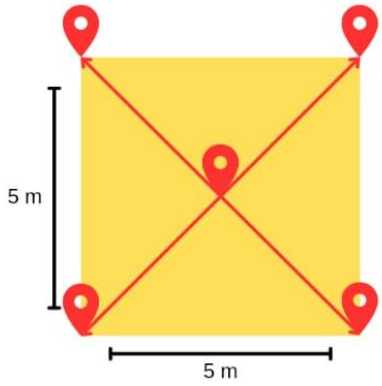

Figure 2. Soil sampling point at harvest time in each plot

Data Analysis. Data on growth and yields of sweet corn and bacteria density were processed using analysis of variance (ANOVA). The multiple comparison test, Duncan's multiple range test (DMRT), was employed once significant differences were detected at an α-value of 5%.

Results and Discussion

Weed identification. Weed identification aims to determine the dominant weed. This is the first step for successful weed control (Mishra & Gautam, 2021). Based on the results of weed identification before sweet corn planting, the number of weed species identified was 18 (Table 1).

Table 1. Summed dominance ratio (SDR) value of weeds before herbicide application

No.	Weed	SDR(%)
	Broadleaf weed	
1	*Ageratum conyzoides*	4.51
2	*Physalis angulata*	2.12
3	*Peperomia pellucida*	1.23
4	*Cleome rutidosperma*	6.49
5	*Alternanthera philoxeroides*	9.36
6	*Tridax procumbens*	2.24
7	*Ipomoea reptans*	5.57
8	*Borreria alata*	0.97
9	*Hedyotis corymbosa*	1.30
10	*Rorippa palustris*	1.12
11	*Eclipta prostrata*	1.61
	Grassy weed	
1	*Echinochloa colona*	16.27
2	*Eleusine indica*	19.38
3	*Bracharia mutica*	2.12
4	*Murdannia nudiflora*	5.40
5	*Cyperus rotundus*	9.32
6	*Digitaria ciliaris*	7.42
7	*Digitaria sanguinalis*	3.58
	Total	100

The land before planting sweet corn was dominated by grassy weeds. Dominant weeds are determined through vegetation analysis by calculating the SDR value (Firmansyah & Pusparani, 2019). The weeds that dominate are the grassy weeds *Eleusine indica* (SDR 19.38%), *Echinochloa colona* (SDR 16.27%), and *Cyperus rotundus* (SDR 9.36%). The dominant broadleaf weed is *Alternanthera philoxeroides* (SDR 9.36%). Weeds dominance on a land influenced by superior physiological characteristics of weeds, such as high germination, pollination capacity, and rapid adaptation to the environment (Anwar et al., 2021).

Table 2. Summed dominance ratio (SDR) of weeds in sweet corn fields aged 15 and 35 DAP

No.	Weed	15 DAP								35 DAP							
		H0	H1	H2	H3	H4	H5	H6	H7	H0	H1	H2	H3	H4	H5	H6	H7
	Broadleaf weed																
1	*Ageratum conyzoides*	9.46	3.05	2.67	34.06	3	4.66	3.62	4.56	18.27	19.09	31.16	33.11	5.78		7.5	
2	*Peperomia pellucida*				8.04												
3	*Cleome rutidosperma*							1.81		1.34	8.33	6.69	6.18				
4	*Euphorbia hirta*	1.41		2.27		2.58	5.16	2.01		2.05							
5	*Scoparia dulcis*	1.54							5.46		5.9	1.37					
6	*Calyptocarpus vialis*	2.77							9.98		3.84						
7	*Pyllanthus urinaria*		2.19	2.03							1.13						
8	*Tridax procumbens*	3.11	4.97				4.47	1.74									
9	*Chenopodium album*	1.61				2.34											
10	*Physalis angulata*									3.29	1.27	3.50	9.39				
11	*Alternanthera philoxeroides*									10.76	7.49	10.53	3.06			4.79	5.68
12	*Eclipta prostrata*	6.96	12.77	14.87		16.09	4.8	15.66		3.89	1.44	1.70					15.50
13	*Parietaria judaica*	5.31	6.64	6.45		7.57		4.5						4.78			
14	*Ipomoea reptans*	2.7	2.4	4.45		8.08	8.95	3.36		1.47							
15	*Sisymbrium officinale*	2								3.99	4.17		1.78	22.04	10.77	12.98	20.09
16	*Cirsium arvense*			2.3								1.62					
17	*Hedyotis corymbosa*	2.29						3.42									
18	*Murdannia nudiflora*										1.36		2.71				
19	*Portulaca oleraceae*							1.82			10.28	7.86					
	Grassy weed																
1	*Echinochloa colona*	8.12	2.29	5.55	7.29	6.21		1.86		2.39	4.43	2.83	12.35	52.34	6.26	23.97	
2	*Eleusine indica*	18.47	34.96	17.37	36.9	15.2	39.57	21.29	48.18	19.22	20.80	22.92	15.82		31.99		19.38
3	*Bracharia mutica*	14.9	5.79	15.66				9.13		10.17						7.26	
4	*Commelina diffusa*	1.75	5.14	4.28				11.07	11.55					15.06		29.40	
5	*Kyllinga brevifolia*	10.07	10.38	22.1	13.72	28.16	32.39	12.45	20.27	5.46	7.83	7.37	15.59		50.97		15.78
6	*Cynodon dactylon*		2.66			3.69				2.82						14.1	
7	*Bracharia eruciformis*					7.08				3.82	2.63	2.46					
8	*Cyperus rotundus*	2.38								6.67							23.56
9	*Digitaria ciliaris*	5.15	6.77					6.26		4.38							
	Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Note: H0 (control), H1 (manual weeding), H2 (paraquat), H3 (glyphosate), H4 (paraquat, atrazine, and mesotrione), H5 (glyphosate, atrazine, and mesotrione), H6 (paraquat, atrazine, mesotrione and nicosulfuron), H7 (glyphosate, atrazine, mesotrion and nicosulfuron).

There were several new types of weeds that did not grow before sweet corn planting, but grew at 15 DAP (21 days after application of paraquat and glyphosate) and 35 DAP (20 days after application of atrazine, mesotrione, and nicosulfuron). These weeds include *Euphorbia hirta, Scoparia dulcis, Calyptocarpus vialis, Pyllanthus urinaria, Chenopodium album, Parietaria judaica, Commelina diffusa, Sisymbrium officinale, Kyllinga brevifolia, Cynodon dactylon, Cirsium arvense, Bracharia eruciformis, Portulaca oleraceae*. There are also types of weeds that appeared before planting, but did not reappear during observation, i.e., *Borreria alata* and *Rorippa palustris*.

The weeds that grew in control and manual weeding were more diverse than in herbicide treatment. At the 15 DAP observation (21 days after the application of paraquat and glyphosate), types of weeds grew in glyphosate treatment fewer than in paraquat treatment. The dominant weeds in sweet corn cultivation land in all treatments were *Eleusine indica* and *Kyllinga brevifolia*. This indicates that both types of weeds show preliminary indications of resistance to paraquat and glyphosate.

Glyphosate is non-selective herbicide that works by inhibiting the activity of enzyme 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) which catalyzes the sixth step in the shikimic acid pathway (Tampubolon et al., 2019). Glyphosate inhibits this enzyme, which stops the shikimate pathway from producing aromatic amino acids like tryptophan, tyrosine, and phenylalanine (El-Mergawi et al., 2025). Glyphosate can translocate within plants, accumulate in roots, and be released into the rhizosphere by the root exudates. Inside the plant, glyphosate may be transported within the plant xylem in the apoplastic pathway or enter the phloem and get transported to metabolic sinks via the symplastic pathway. For both foliar and root uptake, glyphosate translocation may be basipetal or acropetal (upwards and downwards), moving toward various tissues, such as meristems, leaves flowers, and fruits. As glyphosate is stable and not immediately metabolized in many plant species, substantial amounts can be extensively translocated to regions of active growth and accumulate, particularly in young tissues. Glyphosate reaches any actively growing tissue or organ. The physicochemical properties and high solubility of glyphosate in water enable it to be translocated via the phloem to the same tissues that are metabolic sinks for sucrose (Zioga et al., 2022).

Eleusine indica is a weed that grows quickly, especially at high sunlight intensity and is classified as a C4 plant (Correia et al., 2022). This weed flowers all year round, can self-pollinate and can produce up to 140,000 seeds per plant (Tampubolon et al., 2019). The seeds can survive for up to 2 years in a soil depth of 20 cm and have a growth capacity of 79% (Umiyati et al., 2023).

Eleusine indica has resistance to various herbicides, including glyphosate, paraquat, ammonium glufosinate, and ACCase inhibitors (Kurniadie et al., 2023). Repeated applications of herbicides with the same mode of action can select for herbicide-resistant biotypes. *Eleusine Indica* was found to be resistant to glyphosate in research conducted on oil palm plantations in North Sumatera (Tampubolon et al., 2019). Weed genetic diversity, which arises from target-site or non-target-site modifications, is the cause of Eleusine indica's herbicide resistance (Deng et al., 2022).

Kyllinga brevifolia is a perennial weed that grows using rhizome when turf is maintained. Its populations form by germination of seeds, which peaks between 20 and 24 C. Hand pulling or digging is frequently useless when trying to manually eradicate Kyllinga brevifolia from turf grasses. By using rhizomatous growth to regenerate new plants, *Kyllinga brevifolia* are able to escape preemergence herbicides (Westbury et al., 2022).

Weeds grew with post-emergence herbicide treatment at 35 DAP (20 days after application of atrazine, mesotrione, and nicosulfuron) is lower than in the treatment without post-emergence herbicide. Application of atrazine, mesotrione, and nicosulfuron aims to prevent weed resistance after application of glyphosate and paraquat. Atrazine, mesotrione, and nicosulfuron have a different mechanism of action from glyphosate and paraquat, so they can increase the effectiveness of weed control (Arslan et al., 2016; Xu et al., 2022).

The dominant weeds in control treatment, manual weeding, and application of paraquat and glyphosate were *Eleusine indica* (grassy weed) and *Ageratum conyzoides* (broadleaf weed). The dominant weeds in the paraquat, atrazine, mesotrion (H4) and paraquat, atrazine, mesotrion, nicosulfuron (H6) treatments were *Echinocloa colona* and *Commelina diffusa*. In the glyphosate, atrazine, mesotrione treatments, *Eleusine indica* and *Kyllingia Brevifolia* were dominated, while the glyphosate, atrazine, mesotrione and nicosulfuron treatments were dominated by *Eleusine indica* and *Cyperus rotundus*. This indicates that the weed is resistant to post-emergence herbicide.

The synthetic triazine herbicide, i.e., atrazine (6-chloro-N-ethyl-N0-(1-methylethyl)-1,3,5-triazine-2,4-diamine) used to suppress broadleaf and grassy weeds in corn, beans, sorghum, wheat, and sugarcane (Kumar & Singh, 2016; Zhao et al., 2017). Atrazine enters through the roots and is absorbed by the xylem along with water, then inhibit electron transport in photosystem II. Atrazine herbicide poisoning in weeds is characterized by symptoms of chlorosis starting from the edges of the leaves (Cordon et al., 2022).

Mesotrione belongs to the triketone category of herbicides. Mesotrione inhibits ALS (acetolactate synthase) and is efficient against species that are resistant to triazine. This herbicide works by blocking the p-hydroxy-phenylpyruvate dehydrogenase (HPPD) enzyme, which prevents the formation of carotenoid pigments. This can disrupt photosynthesis and result in symptoms such as leaf bleaching and eventual death (Cordon et al., 2022).

Nicosulfuron is a common herbicide used in agriculture, especially corn cultivation, that is effective, safe, and selective at low dosages (Zhang et al., 2020). Nicosulfuron inhibits ALS activity and decreases the synthesis of branched-chain amino acids, including valine, leucine, and isoleucine (Délye et al., 2018). Nicosulfuron also causes chloroplast disintegration and changes plant leaves colour. Photosynthetic pigments and plant protein activities related to photosynthesis were significantly reduced (Xu et al., 2022). This causes inhibition of the plant's electron transport rate, which results in inhibition of ATP and NADPH synthesis (Wang et al., 2021).

According to Ofosu et al. (2023), weeds that may grow and endure following herbicide application are known as herbicide-resistant weeds. Herbicide resistance can be classified into three levels: (i) single resistance, which happens when a weed is resistant to only one group of herbicides and/or one mode of action (like resistance to glyphosate); (ii) cross resistance, which happens when a weed is resistant to one or more groups of herbicides with a similar mode of action (like resistance to imidazolinone and sulfonylurea herbicides, which are both part of the Acetolactate synthase (ALS) mode of action); and (iii) multiple resistance, which happens when a weed is resistant to more than two groups of herbicides and more than two modes of action herbicides (e.g. resistance to Pursuit (ALS) and glyphosate) (Tampubolon et al., 2019).

Growth and Yield of Sweet Corn. Application of glyphosate, atrazine and mesotrione (H5) showed the highest sweet corn plant height values, namely 26.40 cm (2 WAP), 73.17 cm (4 WAP) and 145.20 cm (6 WAP). Herbicide treatment had a significant effect on plant height compared to the control treatment. between the herbicide treatment paraquat, atrazine and mesotrione (H4) and the herbicide treatment glyphosate, atrazine and mesotrione (H5), the effect was not different at plant age of 4 WAP and 6 WAP. Treatments H4 and H5 were able to increase plant height by 23% and 22.5%, respectively, compared to the control. This is because the weed species are not diverse, so there is no tight competition for nutrients even though *E. indica* and *K. brevifolia* are the dominant weeds.

The treatments of glyphosate, atrazine, and mesotrione (H5) also showed the highest number of leaves, namely 4.95 pieces (2 WAP) and 10.85 pieces (6 WAP). The highest number of leaves at the age of 4 WAP was obtained from the paraquat, atrazine a,nd mesotrione (H4) treatments with 7.80 leaves. The herbicide treatment were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at 5% significance level had a significant effect on leaf number compared to the control treatment, but between the glyphosate, atrazine and mesotrione (H5) and paraquat, atrazine, mesotrione, nicosulfuron (H6) treatments the effect was not different. Treatments H5 and H6 increased the number of leaves by 19.88% and 18.78% respectively compared to the control.

The treatments of paraquat, atrazine and mesotrione (H4) and glyphosate, atrazine and mesotrione (H5) showed the highest value of leaf greenness index at 6 WAP, namely 52.15 and 51.92 units. Treatments H4 and H5 increased leaf greenness index by 0.98% and 0.94% respectively compared to the control.

According to Singh et al. (2022), competition between main plants and weeds includes competition in obtaining water, competition in obtaining nutrients because weeds absorb more nutrients than main plants, competition in obtaining light in conditions of sufficient water and nutrients for plant growth, then the next limiting factor is sunlight. Competition between main plants and weeds inhibits plant growth. In general, glyphosate, atrazine and mesotrione (H5) treatment is the best treatment, because plants can compete with weeds for environmental resources, such as space, nutrients, sunlight and water (Lateef et al., 2021; Sawicka et al., 2020). Thus, treatment H5 optimizes the vegetative growth of sweet corn.

The treatment of glyphosate, atrazine and mesotrione (H5) showed the highest value in the variable weight of husked cobs (447.39 g), weight without husks (312.83 g), diameter of husked cobs (65.70 mm), cob diameter without husks (53.49 mm), and cob length (31.73 cm). The H4 treatment (paraquat, atrazine, and mesotrione) showed the highest value for the highest effective corn weight per plot variable (41.87 kg/plot) and corn weight per hectare (16.75 tons/ha), namely increasing the weight by 60.30% compared to the control treatment. Although H5 produced heavier individual cobs, the total yield per plot was greater in H4. This may be attributed to a better plant stand or higher plant survival rate in the H4 treatment, leading to a higher number of marketable cobs per plot.

The rate of weeds development and the physiological and morphological changes to their root systems guarantee that more nutrients are absorbed from the substrate. Weeds consequently become fierce competitor to plants (Sawicka et al., 2020). The growth and development of weeds depend on plant cultivation (Barbaś et al., 2020; Feledyn-Szewczyk et al., 2020; Pszczółkowski et al., 2020), environmental conditions (Ramesh et al., 2017; Varanasi et al., 2016; Vilà et al., 2021), and agricultural practices (Gaweda et al., 2018; Jabran et al., 2017; Nwosisi et al., 2019).

Table 3. Effect of herbicide on growth of sweet corn at 2, 4, and 6 week after planting (WAP)

Treatments	Plant height (cm)			Number of leaves			Leaf greenness index (units)		
	2	4	6	2	4	6	2	4	6
H0	22.95 bc	59.92 b	118.47 b	4.30 c	6.20 d	9.05 c	30.15 b	42.43 b	47.47 c
H1	21.01 ab	65.17 ab	124.75 b	4.30 c	6.70 cd	9.50 c	30.14 b	42.65 ab	48.13 bc
H2	23.40 abc	66.65 ab	120.42 b	4.40 bc	7.05 bc	9.65 bc	31.02 ab	44.35 ab	48.37 bc
H3	22.27 bc	69.65 ab	134.60 ab	4.65 abc	7.15 bc	9.95 abc	33.63 ab	44.93 ab	49.92 abc
H4	25.35 ab	71.55 a	145.72 a	4.75 ab	7.80 a	10.45 ab	32.75 ab	45.77 ab	52.15 a
H5	26.40 a	73.17 a	145.20 a	4.95 a	7.55 ab	10.85 a	33.51 ab	46.13 a	51.92 a
H6	24.42 ab	67.10 ab	136.45 ab	4.70 ab	7.45 ab	10.75 a	43.26 a	44.62 ab	51.03 ab
H7	22.65 bc	64.27 ab	132.65 ab	4.50 bc	7.50 ab	10.40 ab	31.72 ab	43.75 ab	51.07 ab

Note: Numbers followed by the same letter in the same column indicate that not significant based on Duncan's multiple range test at 5%; H0 (control), H1 (manual weeding), H2 (paraquat), H3 (glyphosate), H4 (paraquat, atrazine, and mesotrione), H5 (glyphosate, atrazine, and mesotrione), H6 (paraquat, atrazine, mesotrione and nicosulfuron), H7 (glyphosate, atrazine, mesotrion and nicosulfuron)

Table 4. Effect of herbicide on yields of sweet corn

Treatments	Fresh weight of cobs with husks (g)	Fresh weight of cobs without husks (g)	Cob diameter with husks (mm)	Cob diameter without husks (mm)	Length of cob (cm)	Weight per Effective Plot (kg/plot)	Weight per Hectare (ton/ha)
H0	303.93 c	217.04 c	56.80 d	47.65 c	28.24 c	26.12 b	10.45 b
H1	368.75 abc	255.00 abc	61.02 bcd	50.56 bc	29.91 abc	28.50 b	11.40 b
H2	351.57 bc	250.77 bc	59.44 cd	50.46 bc	28.84 bc	28.87 b	11.55 b
H3	390.90 ab	279.24 ab	63.06 abc	51.84 ab	28.99 bc	33.50 ab	14.15 ab
H4	418.48 ab	293.98 ab	64.12 ab	52.31 ab	31.11 a	41.87 a	16.75 a
H5	447.39 a	312.83 a	65.70 a	53.49 a	31.73 a	35,37 ab	14,15 ab
H6	409.95 ab	282.86 ab	21.23 ab	52.08 ab	31.13 a	40.37 a	11.40 b
H7	399.50 ab	276.09 abc	62.47 abc	51.35 ab	30.51 ab	39.75 a	15.90 a

Note: Numbers followed by the same letter in the same column indicate that not significant based on Duncan's multiple range test at 5%; H0 (control), H1 (manual weeding), H2 (paraquat), H3 (glyphosate), H4 (paraquat, atrazine, and mesotrione), H5 (glyphosate, atrazine, and mesotrione), H6 (paraquat, atrazine, mesotrione and nicosulfuron), H7 (glyphosate, atrazine, mesotrion and nicosulfuron)

Herbicides can suppress growth of weed during the vegetative phase of sweet corn plants by inhibiting the photosynthesis process and damaging the weed chloroplast membrane so that it slowly kills the weeds (Kamdem et al., 2016). The formation of corn cobs is greatly influenced by the nutrients absorbed by plant roots in the soil through fertilization and weed control. Sweet corn plants will have less than ideal cob weight if fertilizers are unavailable (Sidahmed et al., 2024). Cob diameter is influenced by the availability of nutrients absorbed by plants, especially phosphorus and nitrogen (Budiastuti et al., 2023). These nutrients can be utilized optimally if the dominance of weeds can be suppressed, so that the size of the cobs formed is larger and the seed density is full. Both genetic and environmental variables affect corn cob length. Corn cob length is not optimal due to ecological factors dominated by weeds (Aisah et al., 2021).

Figure 3. Corn cobs with husks and without husks

Figure 3 shows the difference in ear size harvested from the control and herbicide application treatment. The glyphosate (H3) and paraquat (H2) treatments showed smaller ear sizes compared to the combination treatment with post-emergence herbicides atrazine, mesotrione and nicosulfuron (H4, H5, H6, and H7). A combination of pre-emergence and post-emergence herbicide treatment is an effort that can be applied currently, because there are weed species that are resistant to single herbicide treatment.

Population density of soil bacteria. Total density of soil bacteria pre-application and post-application of herbicides has different density values. The pre-application soil bacterial density was higher than the post-application herbicide bacterial density (Table 5).

Table 5. Number of bacterial densities pre-application and post-application of herbicides

Bacteria	pre-application (CFU/mL)	post-application (CFU/mL)
Total Bacteria	$10.07x10^{-8}$	$9.55\ x10^{-8}$
Phosphate Solubilizing Bacteria	$9.53\ x10^{-8}$	$9.52\ x10^{-8}$
Rhizobium bacteria	$9.90\ x10^{-8}$	$9.40\ x10^{-8}$
Nitrogen Fixing Bacteria	$9.91\ x10^{-8}$	$9.78\ x10^{-8}$

The population and diversity of soil bacteria have decreased due to the toxic nature of exposure to active herbicide ingredients. According to S. Singh et al., (2020), glyphosate changes soil texture and microbial diversity by decreasing microbial populations and increasing populations of phytopathogenic fungi. Research by Adegaye et al. (2023) shows that paraquat can inhibit the population of microorganisms and the growth of bacteria, actinomyces and fungi in the soil. The research by S. Tyagi et al., (2018) show that there is an inhibitory effect on soil microbes due to herbicide application. The bacterial population in the paraquat and atrazine herbicide treatments was lower when compared to the control. The rate of inhibition of bacterial populations was 13.3%-100%, actinomyces populations 8.6%-100%, fungal populations 7.6%-100%. The inhibitory effect becomes weaker as time increases. The bacterial population in all treatments decreased in the 4th week after application, but increased progressively in the 6th and 8th weeks.

Based on the research results shown in table 6, the herbicide treatment glyphosate, atrazine and mesotrione (H5) showed the highest total density of P-solvent bacteria (9.78 CFU/mL), meaning it increased the total density of P-solvent bacteria by 24% compared to the control. The herbicide treatment glyphosate, atrazine and mesotrione (H5) also showed the highest total density of nitrogen-fixing bacteria (9.52 CFU/mL), meaning it increased the total density of nitrogen-fixing bacteria by 16.8% compared to the control.

Tabel 6. Effect of herbicide application on bacterial population density

Treatments	Density of phosphate solubilizing bacteria (CFU/g)	Density of nitrogen-fixing bacteria (CFU/g)
H0	8.77 b	8.15 b
H1	9.34 ab	8.82 ab
H2	8.67 b	8.93 a
H3	9.02 b	8.91 a
H4	8.88 b	9.02 a
H5	9.78 a	9.52 a
H6	8.78 b	9.19 a
H7	8.85 b	9.22 a

Note: Numbers followed by the same letter in the same column indicate that not significant based on Duncan's multiple range test at 5%; H0 (control), H1 (manual weeding), H2 (paraquat), H3 (glyphosate), H4 (paraquat, atrazine, and mesotrione), H5 (glyphosate, atrazine, and mesotrione), H6 (paraquat, atrazine, mesotrione and nicosulfuron), H7 (glyphosate, atrazine, mesotrion and nicosulfuron)

Glyphosate concentrations over a certain period of time have been shown to increase the abundance of several bacteria, such as Proteobacteria, Bulkholderia, Acidobacteria (Adomako & Akyeampong, 2016; Imparato et al., 2016; Newman et al., 2016). Glyphosate application has no effect or does not reduce microbial biomass over a wide concentration range (Nguyen et al., 2018). Glyphosate herbicide treatment has enhanced microbial activity as a result of certain microorganisms' ability to break down glyphosate and use it as a source of carbon for metabolism (Mesquita et al., 2023).The herbicide treatment glyphosate, atrazine, mesotrione (H5) which resulted in an increase in total bacterial density was thought to be because Atrazine had minimal impact on the relative abundance of different bacterial groupings but no discernible influence on bacterial populations (10–12 phyla, 29–34 genera). Atrazine residue levels over time of the year have a certain influence on the enzyme activity and microbial community population, function, in the cultivated soil layer in the corn area of the Chernozem (Yang et al., 2021).

Conclusion

Application of glyphosate and paraquat can reduce weed diversity in sweet corn plantings. The dominant weeds found at 15 DAP were *Eleusine indica* and *Kyllinga brevifolia*. The effectiveness of glyphosate and paraquat weed control individually decreased at 35 DAP of corn plants, causing the dominance of various types of weeds to increase. Application of post-emergent herbicides atrazine, mesotrione and nicosulfuron has proven to be effective and able to reduce weed diversity, especially *Ageratum conyzoides*. The dominant weed found at 35 DAP in the combination treatment of various active ingredients was *Eleusine indica*. The glyphosate, atrazine, and mesotrione treatments showed the best plant growth and yield. Soil bacterial densities after herbicide application were generally lower than before.

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Cluster analysis to explore morphological variation of banana (*Musa* spp.): A case study in Jember and Lumajang, East Java, Indonesia

Abstract. Banana (*Musa* spp.) is an important horticultural plant with a high diversity in Indonesia; yet, knowledge on the morphological characteristics of indigenous bananas in East Java remains inadequate. This research aims to identify the variation and classify banana cultivars in East Java based on morphological characters. The exploration was conducted using the accidental sampling method. The observations include 15 qualitative and 9 quantitative characters. The statistical methods used in this study include descriptive analysis to evaluate the data, correlation analysis to identify relationships between quantitative characters, and cluster analysis to group banana cultivars based on similarity. In the cluster analysis, Euclidean distance was used as a measure of proximity between banana cultivars, and the average linkage method was applied for grouping. The boxplot assisted in studying important characters of each group. The exploration resulted in 15 banana cultivars with varying morphology. The cluster analysis shows banana cultivars can be divided into four major groups. The first group includes six banana cultivars distinguished by many hands per bunch and a short petiole length. The second group comprises seven banana cultivars with medium leaf length and stem diameter, with Morosebo and Musang being the outliers. The third and fourth groups have only one banana cultivar, Musang, and Agung, respectively. The Musang banana has distinct physical characters, most notably a large stem diameter, whilst the Agung banana has a longer fruit length and wider fruit circumference than others. The coefficient of variation (CV) is moderate, with the fruit length, fruit circumference, fruit stem diameter, and stem diameter all having a CV greater than 47%. Thus, they show promise for further improvement. The banana distinct group based on morphological diversity identified in this study provides valuable information for developing targeted conservation strategies and serves as a base for selecting potential parent cultivars in banana breeding programs.

Keywords: Banana · East Java · Morphology · Variation.

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Introduction

Musaceae, also known as the family of banana, is a group of monocotyledons comprising plants of considerable commercial significance and decorative plants (Jenny et al., 2024). *Musaceae* plants are recognized for their huge, lush foliage and distinct, tropical appearance, making them attractive landscaping choices in warmer areas (Parameswaran & Mamiyil, 2018). Banana is one of the most important plants in the *Musa* genus since it contributes to food security and is a popular fruit worldwide (Rouard et al., 2022). Banana is a staple food crop in many tropical locations. In Africa and South America, banana is grown in huge quantities for both domestic use and export. Overall, the Musaceae family plays an important role in the global agriculture and horticulture industries.

Banana consists of wild and cultivated bananas (Ahmad et al., 2020). Wild *Musaceae* species are diploid, having basic chromosome numbers x = 9, 10, and 11. Cultivated banana is derived from hybridization between different wild banana species and subspecies, *Musa acuminata* and *Musa balbisiana* , leading to genomic groups such as AA, BB, AAB, and ABB (Ahmad et al., 2020; Maseko et al., 2024; Nyine et al., 2017). Cultivated banana, which produces edible fruit, is typically parthenocarpic, sterile, triploid (2n = 3x = 33), and propagated primarily vegetatively. Although certain cultivars are diploid or tetraploid, most cultivars differ in chromosome count due to their distinct wild ancestors (Droc et al., 2022). This unique genomic foundation reflects the evolutionary processes behind the development of cultivated banana.

Building upon this genetic basis, edible banana fruit cultivars are a manufactured genetic complex based on two wild diploid species originating from South-East Asia, *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Maseko et al., 2024). *M. acuminata,* has been proposed to have originated in Malaysia or Indonesia (Nasution, 1991), whereas *M. balbisiana* is found in Myanmar, Sri Lanka, India, Malaya, Papua New Guinea, the Philippines, Thailand, and Indonesia. In Indonesia, *M. balbisiana* is widely distributed in Java and Sulawesi (Sunandar, 2017). According to Sunandar & Kurniasih (2019), Indonesia is a banana distribution center with various cultivars, morphologies, and nutritional content. Banana contains varying levels of vitamins, minerals, and carbohydrates. According to Sahmsiah et al. (2024), many properties of each banana cultivar are influenced by genetic and environmental variables, as well as their interaction.

Due to its extraordinary genetic and morphological diversity, Indonesia plays a vital role in global banana production. In 2022, Indonesia's banana production reached 9.6 million tons, with East Java being the top-producing province at 2.6 million tons. Notably, Jember and Lumajang Regencies contributed considerably to this output (BPS, 2023). Given the substantial banana production in Indonesia, particularly in East Java, the need to preserve and enhance this diversity becomes crucial. To preserve this diversity, characterization based on morphological traits can be used, as this method is relatively simple and quick, allowing it to be applied directly to plant populations and the data to be used as a plant description (Kurnianingsih et al., 2018; Sadiyah et al., 2020; Sari et al., 2024). In addition to benefiting biodiversity conservation efforts, the characterization results can serve as a foundation for applied research in various banana-related disciplines, promoting food security and the sustainability of local genetic resources with high economic potential (Maryani et al., 2020).

The diversity of bananas is important because it has the potential to make significant contributions to conservation strategies, enhance the utilization of banana genetic resources, and improve the sustainability of plant production (De Langhe et al., 2018). Currently, efforts are being made to increase the diversity and availability of genetic resources for genetic conservation, including characterization (Droc et al., 2022). Meanwhile, characterization studies can provide valuable information about banana cultivar kinship. This information is useful in breeding efforts because it aids in the development of new cultivars as well as the identification of closely related substitute cultivars when problems arise during cultivation (Hapsari & Lestari, 2016; Weber et al., 2017). The characterization of banana using morphology has been extensively studied by some researchers (Aquino et al., 2017; Sivirihauma et al., 2017; Adheka et al., 2018; Baysal & Ercisli, 2022; Malikongwa et al., 2022; Soares et al., 2023). Banana research in Indonesia has also

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been conducted (Sumardi & Wulandari, 2010; Lesta et al., 2018; Dewi & Damanhuri, 2019; Maryani et al., 2020; Weihan et al., 2020; Sari et al., 2023; Widyayanti et al., 2024). Although previous studies have explored banana diversity, they often focused on broader regions or limited populations, providing insufficient data on local cultivars in major production areas. In East Java, morphology-based characterization remains limited. This study focuses on characterizing bananas in Jember and Lumajang regencies, addressing gaps in regional morphological research and providing valuable insights into their genetic relationships and breeding potential. Specifically, it aims to explore the superior qualities of local banana plants and deepen understanding of their kinship to support breeding programs. To achieve this, cluster analysis was utilized as a multivariate approach to comprehensively examine morphological traits and relationships.

Materials and Methods

Banana exploration. The banana exploration was carried out in East Java province, i.e., the Jember and Lumajang regencies. The exploration used the accidental sampling method (Eichhorn, 2022), based on information from residents. Banana plants as samples were found in residents' yards and fields. Each cultivar of banana is represented by one individual plant. A total of 24 morphological characters were observed, including 15 qualitative and nine quantitative characters. The morphological characters were based on the banana plant descriptor issued by the International Plant Genetic Resources Institute (IPGRI).

Data analysis. The qualitative character data was analyzed using descriptive analysis, which calculated the proportion of frequency of each morphology category. The quantitative character data was evaluated using descriptive, correlation analysis, and clustering methods. The attribute characters are described using descriptive statistics, including minimum, maximum, mean, and coefficient of variation (CV). The relationship between characters would be displayed by corrplot (R software) using Pearson correlation analysis. Cluster analysis (Rodriguez et al., 2019) was used for banana grouping based on quantitative characters. It used the average linkage method and Euclidean distance with the help of PBSTAT 2.2.1 software (Suwarno et al., 2025). Data transformation was carried out first by dividing the original data of each variable by its standard deviation. This transformation had been suggested as a preliminary step before distance calculation to eliminate the scale effect on classification results (DeCastellarnau, 2018). The boxplot helped to study important characters of each group as a result of cluster analysis.

Results and Discussion

This study found 12 banana cultivars from six districts of Jember, namely Ambulu, Balung, Wuluhan, Silo, Kaliwates, and Sumbersari. Meanwhile, three banana cultivars were found in the district of Lumajang, namely Senduro and Pasrujambe (Figure 1). Thus, the exploration obtained 15 different banana cultivars (Table 1). Several banana cultivars obtained were similar to those explored in the study by Hermanto, et al. (2013) in the Ambon, Seram, Flores, Sumba, and Bali areas, namely Candi, Tanduk, Raja, Susu, and Nangka banana.

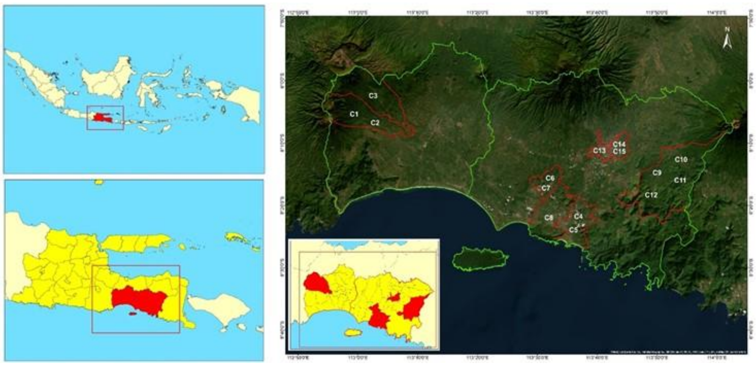

Figure 1. Banana exploration areas in East Java Province

Table 1. Results of banana plant exploration in East Java Province

Number of cultivars	Banana cultivars	Village	District	Regency
C1	Agung	Pasrujambe	Pasrujambe	Lumajang
C2	Cavendish	Pasrujambe	Pasrujambe	Lumajang
C3	Musang	Sari Kemuning	Senduro	Lumajang
C4	Candi	Andongsari	Ambulu	Jember
C5	Janten	Sabrang	Ambulu	Jember
C6	Susu	Balung Lor	Balung	Jember
C7	Raja Nangka	Balung Lor	Balung	Jember
C8	Nangka	Glundengan	Wuluhan	Jember
C9	Sisir/Jari Buaya	Garahan	Silo	Jember
C10	Embug	Sempolan	Silo	Jember
C11	Seribu	Sempolan	Silo	Jember
C12	Rayapan	Sempolan	Silo	Jember
C13	Kepok Makassar	Gebang	Kaliwates	Jember
C14	Awak	Tegal Gede	Sumbersari	Jember
C15	Morosebo	Tegal Gede	Sumbersari	Jember

Identification of qualitative characters of banana. Table 2 showed that the pseudostem color was green-red by 40%, yellowish green by 33.33%, and none of them were dark green or blue. The difference in pseudostem color was due to the metabolic process that produced a color pigment, and each cultivar of banana had a different pigment gene (Dewi & Damanhuri, 2019). The content of anthocyanin pigment was the cause of the variety in pseudostem colors. The anthocyanin pigment content had different biosynthesis pathways between the two ancestors, namely *M. acuminata* and *M. balbisiana*. Bracts, male bud shape, and mature fruit color allow the differentiation between ploidies and banana subgroups (Beaton et al., 2023). Most bananas have red, purple, or violet bracts, however, a few are acyanic (green or yellow). The color of the bracts varies according to the composition of the anthocyanins present, which is unique to each species and subspecies. As a result, taxonomic tools such as anthocyanin were utilized to differentiate taxa. Different species' bracts have been found to contain all six prevalent anthocyanidins (Pazmiño et al., 2001).

Table 2 also shows that the largest percentage of the petiole shape character was a category of wide with erect margins, accounting for 40.0%. Then, the categories of open with margins spreading, straight with erect margins, and margins curved inward were 26.67%, 20%, and 13.33%, respectively. According to Dewi & Damanhuri (2019), *M. acuminata*-derived banana had a petiole growth type with upright sides, while *M. balbisiana* was an inward-curved type. Meanwhile, more than half of the banana varieties had slightly hairy banana bunch stalks. The length of fruit stalks had three categories (long, medium, and short) observed by measuring from the base of the stem to the tip (IPGRI, 1996). In this study, all the bananas explored in the long category were more than 21 cm. The arrangement of ovules had two categories, namely 2 and 4 rows, each with almost the same proportion, nearly 50%.

Table 2. Frequency of 15 qualitative characters in each cultivar of banana

Character	Category	Frequency (%)	Banana cultivars
Pseudostem color	1. Green-yellow	33.33	Candi, Susu, Nangka, Awak, Embug
	2. Medium green	6.67	Sisir/Jari Buaya
	3. Green	6.67	Kepok Makassar
	4. Dark green	0	-
	5. Green-red	40	Raja Nangka, Agung, Cavendish, Seribu, Rayapan, Morosebo
	6. Red	6.67	Musang
	7. Red-purple	6.67	Janten
	8. Blue	0	-
	9. Other	0	-

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Character	**Category**	**Frequency (%)**	**Banana cultivars**
Petiole canal	1. open with margins spreading	26.67	Raja Nangka, Cavendish, Nangka, Sisir/Jari Buaya
	2. Wide with erect margins	40	Candi, Susu, Agung, Musang, Morosebo, Janten
	3. Straight with erect margins	20	Seribu, Kepok Makassar, Embug
	4. Margins curved inward	13.33	Rayapan, Awak
	5. Margins overlapping	0	-
Peduncle hairness	1. Hairless	40	Candi, Susu, Agung, Kepok Makassar, Awak, Janten
	2. Slightly hairy	53.33	Raja Nangka, Musang, Nangka, Sisir/Jari Buaya, Seribu, Rayapan, Morosebo, Embug
	3. Very hairy, short hairs (similar to velvet touch)	6.67	Cavendish
	4. Very hairy, long hairs (>2mm)	0	-
Fruit pedicle length	1. Long (≥ 21 cm)	100	Candi, Susu, Raja Nangka, Agung, Cavendish, Musang, Nangka, Seribu, Rayapan, Kepok Makassar, Awak, Morosebo, Sisir/Jari Buaya, Janten, Embug
	2. Intermediate (11 to 20 cm)	0	-
	3. Short (≤ 10 cm)	0	-
Arrangement ovules	1. Two-rowed	53.33	Raja Nangka, Agung, Cavendish, Nangka, Seribu, Rayapan, Morosebo, Sisir/Jari Buaya
	2. Four-rowed (more or less)	46.67	Candi, Susu, Musang, Kepok Makassar, Awak, Janten, Embug
Male bract shape	1. $x/y < 0.28$ (lanceolate)	6.67	Agung
	2. $0.28 < x/y < 0.30$	0	-
	3. $x/y > 0.30$ (ovate)	93.33	Candi, Susu, Raja Nangka, Cavendish, Musang, Nangka, Seribu, Rayapan, Morosebo, Sisir/Jari Buaya, Kepok Makassar, Awak, Janten, Embug
Bract behavior before falling	1. Revolute (rolling)	46.67	Susu, Raja Nangka, Musang, Nangka, Kepok Makassar, Awak, Morosebo
	2. Not revolute (not rolling)	53.33	Candi, Agung, Cavendish, Seribu, Rayapan, Sisir, Janten, Embug
Bract base shape	1. Large shoulder	26.67	Musang, Kepok Makassar, Janten, Embug
	2. Medium	26.67	Candi, Susu, Cavendish, Awak
	3. Small shoulder	46.67	Raja Nangka, Agung, Nangka, Seribu, Rayapan, Morosebo, Sisir/Jari Buaya
Bract apex shape	1. Pointed	26.67	Raja Nangka, Agung, Rayapan, Morosebo
	2. Slightly pointed	33.33	Candi, Cavendish, Nangka, Seribu, Sisir/Jari Buaya
	3. Intermediate	26.67	Susu, Musang, Kepok Makassar, Awak
	4. Obtuse	6.67	Janten
	5. Obtuse and split	6.67	Embug
Color of the bract external face	1. Yellow	0	-
	2. Green	0	-
	3. Red	20	Sisir/Jari Buaya, Janten, Embug
	4. Red-purple	53.33	Susu, Raja Nangka, Agung, Nangka, Seribu, Rayapan, Kepok Makassar, Awak
	5. Purple-brown	0	-
	6. Purple	13.33	Candi, Cavendish
	7. Blue	0	-
	8. Pink-purple	6.67	Morosebo
	9. Orange-red	6.67	Musang
Fading of color on bract base	1. Colour discontinuing towards the base	26.67	Agung, Morosebo, Janten, Embug
	2. Colour homogenous	73.33	Candi, Susu, Raja Nangka, Cavendish, Musang, Nangka, Seribu, Rayapan, Kepok Makassar, Awak, Sisir/Jari Buaya
Bract scars	1. Very prominent	86.67	Candi, Raja Nangka, Agung, Cavendish, Musang, Nangka, Seribu, Rayapan, Kepok Makassar, Awak, Morosebo, Sisir/Jari Buaya, Janten
	2. Not prominent	13.33	Susu, Embug

Character	Category	Frequency (%)	Banana cultivars
Free tepal of male flower	1. Simple folding under apex	40	Candi, Susu, Raja Nangka, Kepok Makassar, Awak, Sisir/Jari Buaya
	2. More or less smooth	33.33	Cavendish, Nangka, Seribu, Rayapan, Morosebo
	3. Several folding under apex (corrugated)	26.67	Agung, Musang, Janten, Embug
Male flower color	1. White	0	-
	2. Cream	46.67	Susu, Raja Nangka, Cavendish, Nangka, Seribu, Rayapan, Embug
	3. Yellow	33.33	Candi, Musang, Kepok Makassar, Awak, Morosebo
	4. Pink/pink-purple	13.33	Agung, Janten
Stigma color	1. Cream	66.67	Sisir/Jari Buaya, Susu, Agung, Janten, Nangka, Kepok Makassar, Awak, Morosebo, Sisir/Jari Buaya, Candi, Embug
	2. Yellow	0	-
	3. Pink/pink-purple	6.67	Cavendish
	4. Bright yellow	13.33	Seribu, Rayapan
	5. Orange	13.33	Raja Nangka, Musang

Table 3. Minimum, maximum, mean, and coefficient of variation (CV) of the quantitative character of banana

Character	Min	Banana Cultivar	Max	Banana Cultivar	Mean	CV (%)
Fruit length (FL, cm)	6.17	Morosebo	30.00	Agung	12.30	48.85
Fruit circumference (FC, cm)	1.23	Janten	16.60	Agung	8.24	55.33
Peduncle diameter (PLD, cm)	1.23	Janten	7.33	Cavendish	4.34	49.92
Petiole length (PL, cm)	7.50	Morosebo	64.50	Awak	39.56	44.86
Leaf blade length (LL, cm)	76.00	Candi	338.33	Sisir	214.44	33.72
Leaf blade width (LW, cm)	35.00	Rayapan	79.67	Kepok Makassar	62.72	22.2
Pseudostem diameter (PSD, cm)	11.33	Morosebo	77.33	Musang	24.08	70.57
Number of hands per bunch (NC)	2.00	Agung	12.00	Cavendish	7.07	34.02
Pseudostem height (PSH, cm)	109.00	Morosebo	489.00	Kepok Makassar	288.67	40.91

Qualitative characters were only controlled by one or two genes (simple genes) with small environmental influences (Ritonga et al., 2022). For example, the yellow color character in banana is controlled by one gene, namely the ZmPsy1 gene (Paul et al., 2017). The 15 banana cultivars explored in this study show variation in qualitative morphological character (Figure 2). The uniqueness of this morphology could be influenced by different environments and banana genome ancestors such as *M. acuminata* and *M. balbisiana*, and it could be expressed as a unique category (Herwitarahman & Sobir, 2014).

Identification of quantitative characters in banana. Morphological characters can be classified into qualitative and quantitative characters (Serpico, 2020). Quantitative traits, especially leaves, flowers, and stems, are characters of plants that vary and often undergo changes. This can be influenced by the environment and genes that affect physiological processes (Sari et al., 2023). The descriptive analysis of quantitative morphological characters in this study is presented in Table 3.

Table 3 shows that the fruit length ranges from 6.17-30.00 cm, which is not significantly different from the research by Lesta et al. (2018) on 22 banana germplasm from Bangka, where the fruit length ranges from 11.06-32.22 cm. The width and length of the leaves in this study were, respectively, within the intervals of 35.00-79.67 cm and 76.00-338.33 cm. Both variables of the leaves are also not different from the banana from Bangka, where the width and length of the leaves range from 35.03-71.35 and 135-272 cm. However, the range of leaf length from this study is almost twice the range of leaf length of banana from Bangka. Meanwhile, the height of the banana stems from Bangka ranges between 245-645 cm, relatively taller compared to the banana trees in this study.

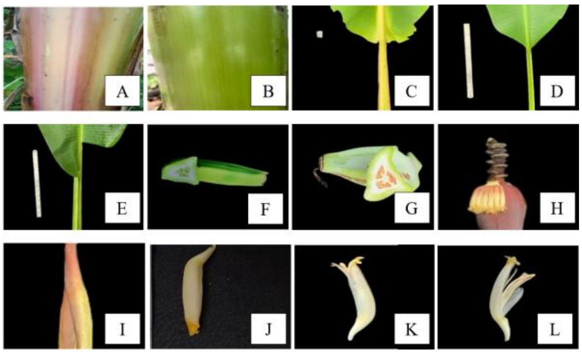

Figure 2. The Qualitative Characters of Explored Banana. Pseudostem Color: Reddish Green (A), Green (B); Petiole Canal: Open with Margins Spreading (C), Wide with Margins Spreading (D), Margins Curved Inward (E); Arrangement of Ovules: Two-Rowed (F), Four-Rowed (More or Less) (G); Male Bract Shape: Ovate (H), Lanceolate (Ii; Free Tepal of Male Flower: More or Less Smooth (J), Simple Folding Under Apex (K), Corrugated (L)

Quantitative traits in banana plants have a certain level of variation. The relative variation of each quantitative character can be measured using the coefficient of variation (CV). The CV provides important information about how consistent or variable the data obtained from various genotypes is, which is crucial in decision-making for selecting superior varieties (Gangappa et al., 2022). The CV value can help in identifying traits with high variability that have the potential for development in plant breeding programs. The smaller the CV of a character, the lower the level of variation of that character, or the more homogenous it is. Conversely, the higher the CV value, the greater the variability (Weihan et al., 2020).

The CV in this study ranges from 22.2% (leaf width) to 70.57% (pseudostem diameter). Meanwhile, for fruit characters, both fruit circumference and fruit length have relatively high CVs compared to other characters, except for stem diameter and fruit peduncle diameter. Compared with the study by Weihan et al. (2020) in 6 sub-districts in the mainland area of Tanjung Jabung Timur Regency, Jambi Province, Indonesia, which produced a CV ranging from 13.05% (fruit length) to 30.02% (number of hands), this study found a higher value of CV.

Variation is essential for plant breeding programs, as it allows breeders to select specific traits and create new varieties with desired characters. By understanding the genetic and environmental factors that influence phenotype variation, breeders can make informed decisions about which plants to cross and which traits to select for future generations. Additionally, studying phenotype variation can help researchers better understand the underlying genetic mechanisms that control important traits in plants, leading to more targeted and efficient breeding strategies (Mackill & Khush, 2018; Upadhyaya et al., 2019).

The quantitative characters of plants are affected by numerous genes as well as environmental factors. The environment has a significant impact on how plants grow. Different planting timings and environmental factors like sunlight, temperatures, and soil properties (humidity, pH, texture) might contribute to variations in quantitative characters (Kurnianingsih et al., 2018).

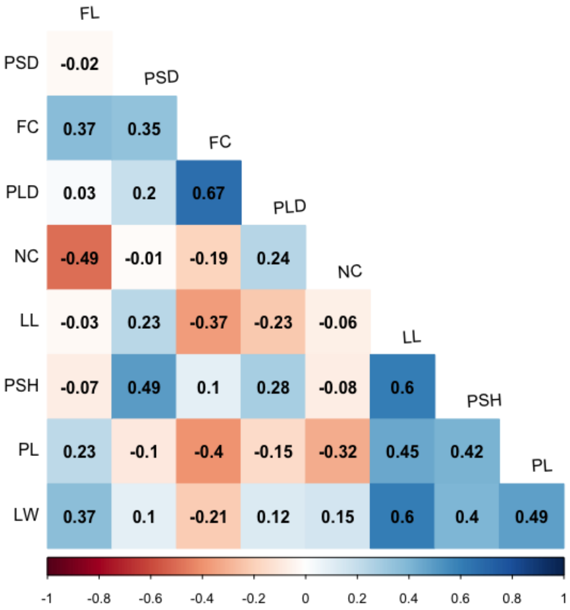

Figure 3. Correlation of quantitative character in banana

Plant breeding programs often use correlation analysis for indirect selection. If vegetative traits, which can be observed earlier, are strongly correlated with main traits to be improved (e.g., production characteristics like fruit number and size), indirect selection can accelerate the selection process. In this study, most correlations between variables were not statistically significant (Figure 3). There was a significant correlation between the fruit circumference and the peduncle diameter (r=0.67, p-value < 0.05), the length and width of the blade of leaves (r=0.6, p-value < 0.05), as well as the leaf blade length and the pseudostem height (r=0.6, p-value=0.05). Based on the correlation analysis, indirect selection was not recommended in the banana breeding process, especially the cultivars included in this study.

The clustering of banana cultivars by quantitative characters. The explored bananas were grouped based on quantitative characters presented in the circlized dendrogram in Figure 4. Based on the dendrogram, 15 cultivars of banana were grouped into four main groups. Group 1 consisted of six banana cultivars, Group 2 had seven banana cultivars, and Groups 3 and 4 consisted of only one banana cultivar. The distance between groups used a value of cophenetic distance close to 4.3. The boxplots for each group on each quantitative character are created to find out the main character of each group (Figure 5).

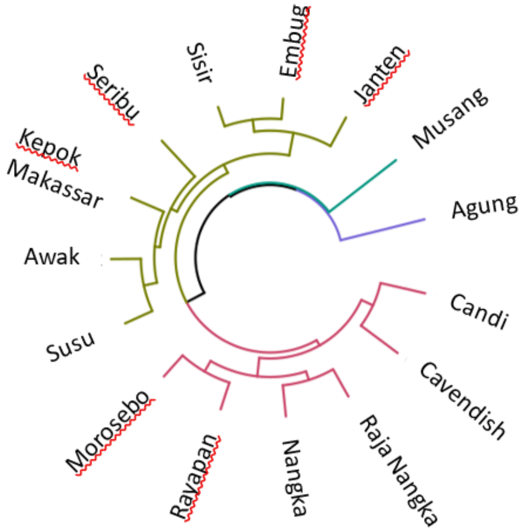

Figure 4. Circlized dendrogram of cluster analysis of 15 banana cultivars based on nine quantitative characters using euclidean distance from transformed data and the average linkage method

Group 1 consisted of Banana Candi, Cavendish, Raja Nangka, Nangka, Rayapan, and Morosebo. The closest distance in Group 1 included Morosebo and Rayapan, with a cophenetic distance of 1.73. All cultivars of banana in Group 1 had the same ovule arrangement, which is two rows, except for the Candi banana. The main character of Group 1 was a high value of the number of hands per bunch and a low value of petiole length. The variance of the leaf blade width was high, indicated by the box size on the boxplot being relatively long. It could be interpreted that Group 1 had a leaf width size that varied in value.

Group 2 consisted of Susu, Awak, Kepok Makasar, Seribu, Sisir, Embug, and Janten banana. The stigma color of all bananas in Group 2 was cream, except the stigma color of the Seribu banana was bright yellow. Group 2 was also characterized by high values of leaf blade length and petiole length relative to the others, as well as moderate pseudostem diameters.

Groups 3 and 4 consisted of only one cultivar of banana, namely Musang and Agung banana, respectively. The prominent character in Group 3 was the pseudostem diameter, which was larger than the others, and the peel color when ripe was red. Meanwhile, the Agung banana (Group 4) had a much higher length and circumference of fruit than other bananas, as well as the lowest number of hands per bunch, which was only two hands per bunch.

Cluster analysis is grouping cultivars based on their similarities. The more character differences, the lower the similarity value, indicating a greater distance in the kinship relationship between the compared cultivars. The closer the position of two cultivars in the dendrogram, the greater their similarity. Banana cultivars with a low similarity coefficient are well-suited to be used as parents in crosses. The smaller the genetic similarity between the parents used, the greater the chance of obtaining hybrids with relatively high genetic variation, which will produce individuals with higher heterozygosity (Rao & Hodgkin, 2002).

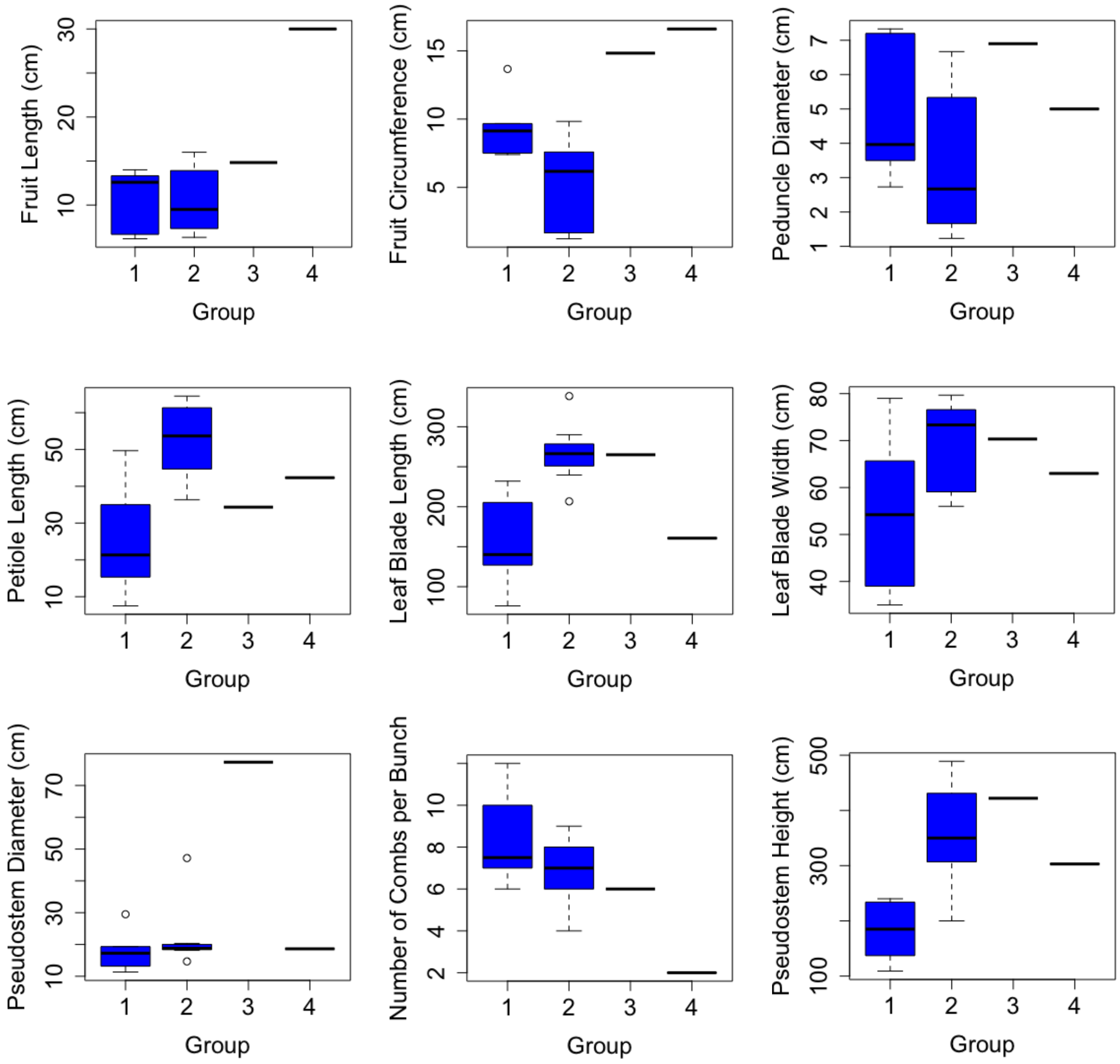

Figure 5. Boxplot per group for each quantitative character

The research of Widyayanti et al. (2024) on banana germplasms from Yogyakarta produced four groups, which consisted of 3-12 cultivars per group. The main characters of each group were the appearance of the upper surface of the leaves, the presence of male flowers, and the existence of hermaphrodite flowers, which had a more dominant visualization color than the others. Meanwhile, research on 22 banana germplasms in Bangka by Lesta et al. (2018) showed that the variability of banana in Bangka was relatively wide. High variability indicated the diversity of germplasm and the high effectiveness of selection. If the genetic variability was narrow, the selection could not be carried out because the observed population was uniform (Salgotra & Chauhan, 2023).

Banana populations with narrow variation have to broaden. Broadening the crop's genetic base while maintaining quality characteristics and enhancing production performance is critical. Furthermore, the huge genetic group will be useful for future genomic selection research in the crop, as well as for profiling conservation methods (Arvanitoyannis et al., 2008).

Germplasm that has large variations is a source of genes for the traits of high yield, resistance to pests or diseases, early maturity, and other good traits. Plants in the same group, if used as parents for crosses, will produce offspring with low genetic variation, while crosses between groups will produce high genetic variation. To determine the role of genotype and environment can be calculated through the phenotype diversity in a generation (Sari et al., 2023). However, genetic limitations such as sterility and parthenocarpy in bananas make traditional breeding difficult and time-consuming. Therefore, modern molecular breeding technology become an inevitable requirement for genetic improvement of bananas and breeding of new varieties (Miao et al., 2025).

Conclusion

Based on the observations of banana varieties from the exploration in Jember and Lumajang regencies, it can be concluded that these bananas have moderate genetic variability, making the selection of germplasm quite effective in supporting the local banana breeding program. Selection should be conducted directly because the correlation analysis results show low to moderate values; thus, indirect selection is not recommended. In general, the banana resulting from the exploration can be grouped into four groups, each with distinct prominent characters. This research is expected to provide insights that can be used in plant breeding programs, conservation of germplasm, and management of local genetic resources with high economic potential.

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Agronomic performance and stability of Padjadjaran hybrid maize in different agroecosystems

Abstract: The development of high-yielding hybrid maize is a strategic step to address global food demand amidst climate change. This study evaluates the agronomic performance of 14 maize genotypes—10 newly developed F1 hybrids and 4 commercial checks—under two planting densities (95,000 and 55,000 plants/ha) across three agroecosystems (lowland, midland, and highland). The objectives were to identify superior and adaptive genotypes and analyze genotype × environment interactions using AMMI and GGE biplot methods. Genotype G12 exhibited the highest yield (338.4 g/plant) at high density, especially in the highland, but showed poor stability. In contrast, G5 demonstrated consistent performance and stability across environments. This study highlights G5's potential for broad adaptation and provides insights for breeding programs targeting maize productivity in diverse agroecosystems.

Keywords: AMMI · GGE · Planting density · Environment · Stability · Regression

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Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops for global food security, providing a significant portion of caloric intake worldwide (Desoky et al., 2021; Luo et al., 2022; Ma et al., 2024). In Indonesia, however, maize production is challenged by a 39.18% decline in harvested area and a 13.4% drop in output due to land-use change, urbanization, and climate variability (Directorate General of Food Crops, 2024). To maintain national food security, it is critical to develop high-yielding and environmentally adaptable hybrids.

Modern hybrids are designed for resource efficiency and better performance under dense planting, but this approach introduces inter-plant competition for light, water, and nutrients (Omar et al., 2022; Ruswandi et al., 2021; Kamara et al., 2021). Therefore, determining the optimal density and assessing phenotypic stability under multiple environments is essential (Jaikumar et al., 2021; Rizzo et al., 2022; Fadhillah et al., 2021).

In plant breeding, yield stability is an important parameter determining the success of genotype adaptation to environmental variations (Pramitha et al., 2022; Ruswandi et al., 2023). This yield stability is often influenced by the genotype-environment interaction (G × E) (Mansour et al., 2018). According to Ruswandi et al. (2022), differences in the growing conditions result in varying responses for each genotype tested. Analysis methods such as Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype plus Genotype-by-Environment Interaction (GGE) biplot have proven effective in evaluating G × E interactions and identifying genotypes with high stability (Wicaksana et al., 2022). This method clearly visualizes the relationship between genotypes and environments and helps select suitable genotypes for various agroecosystem conditions (Vaezi et al., 2019). AMMI analysis is widely used to evaluate the yield of hybrid maize in multi-environment field trials (Mohammed, 2020).

This study aims to (i) evaluate the agronomic performance of new Padjadjaran hybrid maize across agroecosystems and densities, (ii) analyze genotype, environment, and G × E effects on yield, and (iii) identify genotypes with high yield and stability using AMMI and GGE biplot analysis. This research offers practical implications for breeding climate-resilient maize suited to Indonesia's varied topography.

Materials and Methods

This research was conducted in three locations with different agroecosystem characteristics, namely Bojongsoang (lowland), Arjasari (midland), and Ciwidey (highland). The experiment used 14 genotypes comprising 10 new F1 hybrids and four superior commercial hybrids as comparisons. A split-plot design was used, with the main plot being planting density (95,000 plants/ha and 55,000 plants/ha) and the subplot treatment being genotype. Each treatment was repeated three times.

Observations included plant height, stomatal conductance, leaf chlorophyll, leaf area index, and weight per plant. Data were analyzed using combined ANOVA to identify the effects of genotype, environment, and G × E interaction using the PBSTAT statistical software (Maulana et al., 2023). Phenotypic stability parameters, such as regression standard deviation (S^2di) and regression coefficient (bi), were analyzed based on the approach developed by Eberhart & Russell (1966). Significant differences between the value of bi and one were tested using the t-test, while the deviation S^2di was tested using the F-test.

The AMMI (Additive Main Effects and Multiplicative Interaction) model is applied according to the method described by Gauch (2006). The AMMI Stability Value (ASV) is calculated using the formula developed by Purchase et al. (2000). Additionally, the AMMI and SREG (Site Regression) models are used to generate GE and GGE biplots, which illustrate the genotype and environment interactions. The first two principal components (PCA) visualize the relationship between the evaluated genotype and environment (Kempton, 1984).

Results

ANOVA. Combined analysis of variance shows that the main effects of planting environment, planting density, and genotype factor significantly influence all observed characters (Table 1). Variation in rice yield is influenced by genotype by 35.0% of the total sum of squares, while the growing environment and planting density contribute 29.6% and 12.1%, respectively. Furthermore, most of the agronomic traits analyzed were significantly influenced by the two-way and three-way interactions between the

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growing environment, planting density, and genotype. These results are also consistent with Luo et al. (2015) and Shojaei et al. (2022), where the genotype, location, and interactions were statistically significant.

Agronomic Performance. The agronomic performance of ten developed hybrid maize and four commercial hybrids was tested at two planting density levels in three environments, as shown in Figure 1. All observed traits showed significant variation among hybrids in different environments and planting densities. The Ciwidey environment provided the best performance for plant height, stomatal conductance, and seed yield parameters, with respective values of 42%, 12%, and 22.4%. Meanwhile, for the leaf chlorophyll and leaf area index parameters, the best performance was observed in the Arjasari environment with values of 20% and 138%, respectively.

High planting density also increases plant height, leaf area index, and yield by 19.5%, 94%, and 25.9%, respectively. Plant height ranged from 40.4 to 235.9 cm, with hybrids G14, G9, G12, G4, and G3 showing the best performance in Ciwidey, while G11, G4, G7, G13, and G2 excelled in Arjasari (Figure 1a). The leaf area index ranges from 1.21 to 11.7, with hybrids G3, G4, and G1 excelling in Arjasari and G6, G2, and G14 in Ciwidey (Figure 1d); as for the parameters that perform better at low density, such as stomatal conductance and leaf chlorophyll, where the values are 28% and 1% respectively. Stomatal conductance with hybrids G14, G7, and G3 was best in Arjasari and hybrids G2 and G10 in Bojongsoang (Figure 1b). Meanwhile, the chlorophyll values with the G14 and G12 hybrids had the best values in Arjasari and Bojongsoang.

Genotype G12 recorded the highest yield (338.40 g) at high planting density in all locations, although its stability was low. On the other hand, genotype G5 showed the highest stability with a moderate yield (251.12 g) (Figure 1e).

Stability Parameters. The phenotypic stability of the 14 tested hybrid corn has been calculated using the Eberhart and Russell method (Eberhart & Russell, 1966). The results of the combined regression analysis show that the G × L (linear) component has a highly significant influence. In contrast, L + G × L and the agro-environment (linear) do not show a significant influence (Table 2). This emphasizes the importance of linear (bi) and non-linear (s^2di) sensitivity to the expression of agronomic traits (Omar et al., 2022). As shown in Table 3, the regression coefficients (bi) among hybrids range from -0.45 (G6) to 2.91 (G7), indicating the presence of genetic diversity in the regression response. The deviation from regression (s^2di) ranges from -366.43 (G5) to 5306.66 (G12).

Table 1. Combined analysis of variance of 14 hybrid corns at two planting density levels and three planting environments on yield and its characteristics

Source of Variation	df	Plant height	Stomatal conductance	Leaf chlorophyll	Leaf area index	Seed weight
Replication (U)	2	297.97 ns	15236.7 ns	123.54 ns	0.0045 ns	1763.37 ns
Genotype (G)	13	3217.01**	31739.4 ns	445.20**	4.0456**	41643.97**
Error a	26	127.85	975432.5	67.20	0.5437	717.40
Populations (P)	1	47498.11**	500537.1**	76.43 ns	467.90**	48563.90**
Environment (L)	2	110291.89**	503418.1**	1488.52**	231.46**	54790.38**
G × P	13	1175.26**	44187.4**	56.67ns	1.98**	10850.65**
G × L	26	1350.89**	55518.6**	167.46**	2.55**	10982.97**
P × L	2	4802.07**	204498.0**	1012.92**	67.85**	6991.44**
G × P × L	26	695.79**	49900.18**	157.72**	1.73**	5405.97**
Error b	140	136.22	19428.68	64.04	0.45	1462.73
Total	251					

Notes: df was degree of freedom; ** was significant at $p < 0.05$; ns was not significant

Hybrids with a bi value greater than one (bi > 1) and a non-significant s²di, such as G5 and G11, adapt well to supportive agronomic environments, including planting density, planting environment, and other inputs. On the other hand, hybrids with a bi value less than one (bi < 1) and a non-significant s²di, such as G4, show better suitability in less optimal environmental conditions. Hybrids with a bi value close to one and a non-significant s²di, such as G4, show consistent performance stability across various agronomic environments (Table 3). Based on Breese (1969), genotypes with a regression coefficient more significant than one adapt better to favorable environmental conditions, while genotypes with a regression coefficient less than one tend to be more suited for less favorable environmental conditions.

Based on the analysis of the regression coefficient values (bi) and the deviation from the regression (s²di), the most superior and highly stable hybrid is G5. This hybrid has excellent potential to support maize breeding programs in increasing grain yield under high planting density conditions in the three tested growing environments.

(a) (b) (c) (d) (e)

Figure 1. Agronomic performance of 14 hybrid maize genotypes under two planting densities across three agroecosystems: (a) Plant Height (cm), (b) Stomatal Conductance (mmol m⁻² s⁻¹), (c) Leaf Chlorophyll (SPAD), (d) Leaf Area Index (unitless), (e) Seed Weight per Plant (g)

AMMI Analysis. The results of the AMMI analysis revealed that the genotype factor (G), agroecological environment (L), and the interaction between the two (G × E) have a significant influence on the yield (Table 4). The contribution of the sum of squares for each is 46.66% for genotype, 14.75% for environment, and 37.39% for GEI. Visualization through the AMMI1 biplot shows that the G14 hybrid recorded the highest average yield, followed by G12, G5, G13, and G9. Among the groups, G14, G13, G6, and G8 have the lowest first principal component (PC1) values (Figure 2a). G7 showed the highest PC1 value of all the hybrids tested, while G14 had the lowest PC1 value (Figure 2, Table 4). Some hybrids showed specific adaptation to the agroecological conditions of Env5 (low planting density in Ciwidey) and Env1 (low density in Arjasari), compared to other environmental conditions. The Env5 environment produced the highest yield with a PC1 value close to zero, indicating minimal interaction. On the other hand, the highest PCA value was recorded in Env4 (high planting density in Bojongsoang).

The Env3 environment (low planting density in Bojongsoang) and Env4 (high planting density in Bojongsoang) are unstable and more responsive due to their distance from the original location, whereas Env6 and Env2 are less responsive. Similarly, in AMMI2, hybrids G1, G10, G11, G4, and G3 are more stable because they are located near the original site (Figure 2b). On the other hand, hybrids G14, G12, G7, G9, and G2 are far from the original site. Moreover, the environments Env5, Env2, and Env6 are unstable and more responsive due to their distance from the origin.

AMMI shows that genotypes G1, G4, and G10 are the most stable under various environmental conditions.

Table 2. Combined variance regression analysis for the yield of 14 hybrids in six planting environments

Model	83	426860	5143
Genotype (G)	13	173.56	13882 **
L + G × L	70	246391	3520
Environment (linear)	1	57369	57369
G × L (linear)	13	61487	4730 *
Pooled Deviation	56	127534	2277
Pooled Error	156	64954	416

Notes: ^{ns} was not significant; ** was significant at $p < 0.01$; * was significant at $p < 0.05$; df was degree of freedom

Table 3. The stability parameters of 14 hybrid corn were evaluated for Plant Seed's weight at two planting densities and three planting environments

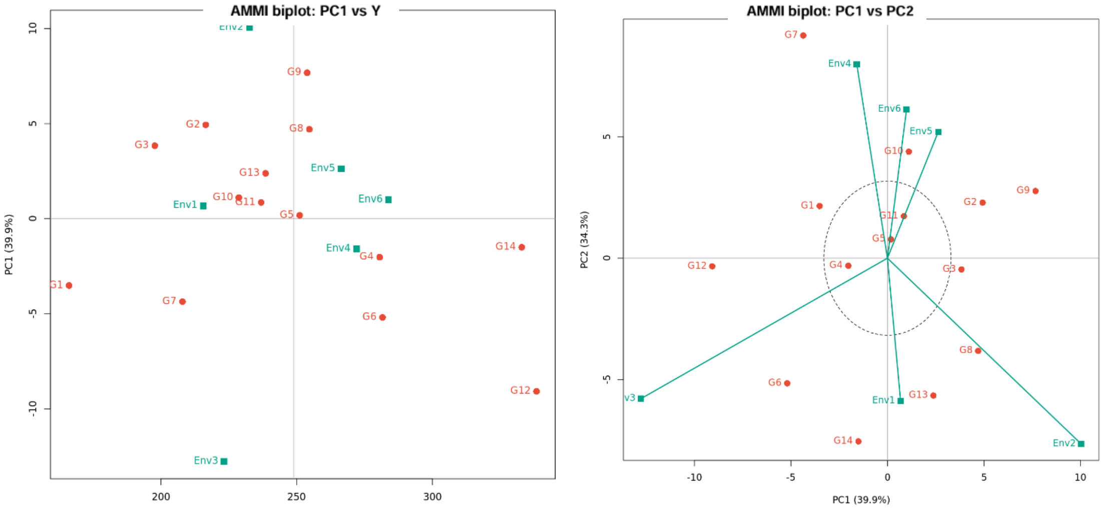
Genotype	Mean (g_i)	b_i	S^2_{di}	ASV	Rank
G1	166.12	1.45	1060.01**	4.62	5
G2	216.48	1.95**	1119.95**	6.18	6
G3	197.71	0.93	857.46*	4.49	4
G4	280.59	0.78	211.67	2.38	2
G5	251.12	1.25	-366.43	0.80	1
G6	281.64	-0.45**	1926.35**	7.94	9
G7	207.91	2.91**	2824.63**	10.48	13
G8	254.67	0.03**	3036.29**	6.67	11
G9	253.86	1.95**	3246.36**	9.34	10
G10	228.68	2.39**	313.66	207.06	7
G11 (Bisi 2)	236.91	1.25	451.48	71.45	3
G12 (Pertiwi 3)	338.40	0.30*	5306.66**	262.73	12
G13 (NK 212)	238.54	-0.30**	798.22*	215.64	8
G14 (Bisi 77)	332.95	-0.43**	5267.98**	357.41	14

Notes: * was Significant at $p < 0.05$; ** was Significant at $p < 0.01$; g_i was the mean of genotype; b_i was the regression coefficient; S^2di was the ean square deviation from linear regression; ASV was AMMI stability value.

Table 4. AMMI analysis of yield variability (ton ha^{-1}) from 14 hybrid corn in six planting environments

Source of Variation	df	Sum of Squares	Mean Square	Percentage
Environment (L)	5	164.57	32.91**	14.75
Replication (U)	12	13.52	1.12 *	1.21
Genotype (G)	13	520.68	40.05 **	46.66
G × L	65	417.21	6.24 **	37.39
PC1	17	200.31	11.78 **	17.95
PC2	15	112.80	7.50 **	10.11
PC3	13	79.15	6.08 **	7.09
PC4	11	16.38	1.48 **	1.47
PC5	9	8.56	0.95 ns	0.77
Residuals	156	77.46	0.49	
Total	95	1115.98		

Notes: ^{ns} was not significant; ** was significant at $p < 0.01$; * was significant at $p < 0.05$; df was the degree of freedom.



a b

Figure 2. AMMI1 (a) and AMMI2 (b) biplots of 10 developed hybrid corn and four commercial checks (G1–G14) evaluated in six agro-environments (Env1-Env6)

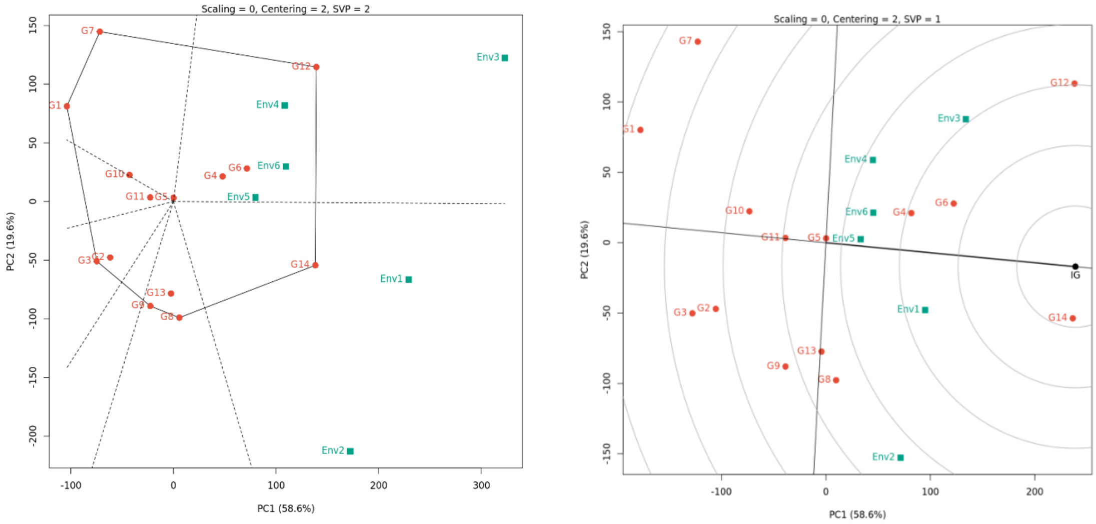


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Figure 3. Distribution plot (a) and comparison graph (b) of the GGE biplot for the yield of 14 hybrid corn (G1-G14) in six environments (Env1-Env6). The horizontal black line directs the tested hybrids towards higher yields, and the other vertical black line crossing the origin of the biplot indicates stability

GGE Biplot. The angle between the environmental vectors indicates the association of genotypes in an environment. The relative length of the vectors in the biplot reflects the proportion of variability associated with each variable. When the angle between two vector properties is less than 90 degrees, it indicates a positive correlation between the two variables. On the other hand, if the angle is more than 90 degrees, it indicates a negative correlation. In addition, a 90-degree angle between two vectors indicates no significant correlation between the two variables (Al-Naggar et al., 2020). The GGE biplot identifies the Env5 environment (Ciwidey with low planting density) as the most representative environment for evaluating hybrid corn, with a high PC1 value and a low PC2 value. Additionally, Env6 (Ciwidey with high planting density) is a favorable environment, but Env1 (Arjasari with low planting density) and Env2 (Arjasari with high planting density) are unfavorable environments with environmental stress.

The GGE biplot graph for the SREG model is illustrated in Figure 3b. The G5 corn hybrid is stable with a GE close to zero. This indicates high yields with the best performance in all agro-environments. In addition, the G12 hybrid is also desirable and closer to the ideal hybrid.

Discussion

This study evaluates 10 new corn hybrids and four superior commercial hybrids at two different plant density levels in three locations (Arjasari, Bojongsoang, and Ciwidey). Analysis of variance was applied to assess the impact of genotype, environmental factors, and their interaction on the agronomic characteristics and quality of hybrid corn (Katsenios et al., 2021). The results of this study confirm that maize genotypes have varying responses to planting density and environmental conditions, consistent with previous research by Sun et al. (2023). Plant density is a factor that influences the survival chances of plants and the efficiency of other input usage. With optimal density, each plant has a better chance to thrive and compete for the necessary resources (Huang et al., 2021).

The values of stomatal conductance, leaf chlorophyll, and leaf area index are also influenced by density treatments, and each genotype produces different responses. Of course, the values of these parameters show specific and stable results across different planting densities. The higher the value of these parameters, the better the plants perform metabolism, especially photosynthesis (Soleh et al., 2020). El Shamey et al. (2022) and Sandhu & Dhillon (2021) reported that the latest hybrid corn is designed for better tolerance to high planting density, which increases yield per unit area. However, the increase in density also heightens competition among plants, which can reduce resource use efficiency. This is evident in the G12 genotype, which, despite recording the highest yield, shows a high dependence on specific environmental conditions, as determining the optimal planting environment becomes a crucial aspect in supporting maize production efficiency, especially in the face of current climate change dynamics (Han et al., 2022).

On the other hand, the G5 genotype shows high yield stability across various environments. This stability can be attributed to better phenotypic adaptation to environmental variations, as Breese (1969) explained. Genotypes with regression coefficient values close to one tend to have better stability, as they can maintain consistent yields despite environmental changes, as in the study by Ma et al. (2020), which explains that the latest hybrids have parameter values that adjust when planting density increases. The phenotypic variability of hybrids developed in various agro-environmental conditions can enable maize breeders to study the genetic potential of hybrids to enhance maize productivity (Omar et al., 2022).

AMMI and GGE biplot analysis provides information about genotype and environment interaction. AMMI is considered significant in evaluating the stability of crop yields under various environmental conditions and in determining the optimal environment for all analyzed genotypes (Agahi et al., 2020). AMMI results show that genotypes G5, G4, and G11 are the most stable, while the GGE biplot identifies the Ciwidey environment as the most representative location. These results are consistent with the research by Gauch (2013), which states that the AMMI and GGE biplot methods are very effective in evaluating the stability and adaptation of genotypes in various environments.

However, this study has limitations, including the lack of evaluation of the impact of pests, diseases, and other agronomic input factors, such as fertilizers and irrigation. Further research needs to include these factors to provide a more comprehensive picture of the performance of maize genotypes in the field.

Conclusion

Analysis shows a highly significant genetic difference among the 14 corn hybrids studied. The evaluated yield characteristics were greatly influenced by the location and plant density. The increase in plant density improved the seed yield of all evaluated hybrids at the Bojongsoang and Ciwidey locations, except for Arjasari. Hybrid corn showed varying responses to the tested agro-environmental conditions. Overall, the G12 and G14 hybrids showed the highest seed yields and attributes in various agro-environments compared to other hybrids. Combined regression analysis, AMMI, and GGE can be used to identify stable maize hybrids across various environments. Stability analysis shows that hybrids G5, G4, and G11 are stable and desirable hybrids. Therefore, these hybrids are recommended for further inclusion in the corn breeding program to increase corn production.

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A review on detection of drought stress in tea plants through morphological, physiological, and biochemical approaches

Abstract. Tea plants (*Camellia sinensis*) are important commodities with high economic value, but their production is greatly affected by environmental stresses such as drought and extreme temperatures. Global warming in recent years has led to extreme weather events and an increase in the earth's temperature, which also causes drought. Drought stress is one of the most significant abiotic factors affecting crop productivity. However, despite significant progress, there remains a notable gap in research, particularly the lack of integrated studies that combine morphological, physiological, and biochemical indicators for early and precise detection of drought stress in tea plants. To identify and mitigate the impact of this stress, a comprehensive approach is needed that includes morphological, physiological, and biochemical aspects. The morphological approach includes changes in leaf structure, stomatal size and number, and root growth patterns. From a physiological perspective, plant response to drought can be seen through measurements of transpiration rate, leaf water potential, and photosynthetic capacity. On the biochemical side, the accumulation of compounds such as proline, antioxidant enzymes, and stress-related hormones, such as abscisic acid, plays a crucial role in plant adaptation to drought conditions. This review summarizes the latest findings related to these indicators by analyzing relevant articles published between 2007 and 2024, obtained through reference searches on Google Scholar and academic databases such as Scopus, EBSCO, and Clarivate. The articles were further analyzed using descriptive methods. This review aims to summarize the latest findings related to these indicators and identify the most effective methods for detecting drought stress in tea plants. A deeper understanding of the tea plant's response to drought through these three approaches is expected to provide a solid basis for developing better drought management strategies to maintain tea plant productivity amid increasingly extreme climate change

Keywords: Abiotic stress · Antioxidant · Climate change · Global warming · Tea

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Introduction

Drought is a climate disaster that occurs under most climatic conditions and can have considerable economic, social, and environmental impacts (Meng et al., 2023). In recent decades, drought caused by rapid warming has deeply affected the global climate. Global climate change will lead to new combinations of temperature and precipitation patterns. Extreme precipitation events become more common by the late 21st century due to anthropogenic warming, particularly in high altitudes; however, the specific responses of high-altitude species are mostly unknown(Zhu et al., 2020) , especially for tea.

Tea plants, as evergreen crops, are highly sensitive to drought stress, which negatively impacts their growth, yield, and quality. According to a report from the International Tea Committee (International Tea Committe (ITC), 2024)), some tea-producing countries experience a 15-30% drop in yield during extreme drought seasons. In India, for example, tea production in Assam and Darjeeling fell by almost 20% in the summer of 2021 due to extreme weather (Food Agricultural Organization, 2022). Climate change is also causing some previously suitable lands for tea cultivation to become unsuitable. A study conducted by Ahmed et al. (2020) shows that in Kenya, it is estimated that about 26% of tea plantation areas will lose their suitability for high-quality tea cultivation by 2050. Similarly, climate model simulations in Indonesia project a reduction in the optimal area of tea cultivation in West Java by 12-18% if temperatures continue to increase by more than 2°C (Fatawa et al., 2024). Climate change is also causing shifts and uncertainty in harvest seasons. For example, green and black tea production experienced significant harvest irregularities in Sri Lanka, making production planning and marketing difficult(Srilanka Teaboard, 2023) . A similar situation was reported in some (Food Agricultural Organization, 2022) tea plantations in West Java, where the timing of young leaf flush became erratic, resulting in supply irregularities (Badan Litbang Pertanian., 2022). Furthermore, changes in temperature and humidity affect the content of secondary metabolites such as catechins, teaflavins, and tearubigins that determine the flavor and aroma of tea. Research by (Han et al., 2018) showed that high temperatures and drought can reduce catechin levels by up to 25%, leading to a decrease in the sensory quality and selling value of tea.

This review summarizes recent research on physiological, biochemical, and molecular responses of tea plants to drought stress. Tea plants are susceptible to changes in environmental conditions, such as fluctuations in temperature, humidity, light intensity, and water availability. Non-optimal environmental conditions can cause stress to the plant, which in turn affects its growth, development, and productivity. Environmental stresses can be divided into several types, including abiotic stresses (such as drought, salinity, extreme temperatures, and light) and biotic stresses (such as pathogen and pest attacks). Each type of stress can trigger specific responses in plants, either morphologically, physiologically, or biochemically. One of the stresses that often hits tea plantations is drought stress, which can cause a decrease in water content in plant tissues, changes in leaf structure, and accumulation of osmoregulatory compounds (Qian et al., 2018)

Drought stresses can be characterized by extreme temperatures, such as high temperatures, which affect key enzymes in the photosynthetic process(Sun et al., 2022) . Increased light intensity causes a decrease in chlorophyll and the yellowing of tea leaves (Zhang et al., 2023). Plants adapt to drought stress in their environment through drought escape, drought avoidance, drought tolerance strategies, or a combination of all three (Yadav & Sharma, 2016) By plants, these strategies are packaged in various forms of morphological, physiological, biochemical, and molecular adaptations as a unified plant adaptation system in response to water deficits in the environment (Zlatev, 2012).

Drought stress is the inevitable factor that exists in various environments without recognizing borders and with no clear warning, thereby hampering plant biomass production, quality, and energy. It is a key important environmental stress that occurs due to temperature dynamics, light intensity, and low rainfall (Seleiman et al., 2021). Drought is a serious threat to tea plantations, significantly affecting tea shoot production. The negative impact of these factors will cause a decrease in the water potential of plant cells, resulting in osmotic stress. Osmotic stress causes oxidative damage and involves the formation of reactive oxygen species (ROS) in plant cells, which then causes membrane damage(Nour et al., 2024). The production of ROS in plant cells displays both detrimental and beneficial effects. However, the exact pathways of ROS-mediated stress alleviation are yet to be fully elucidated (Sachdev et al., 2021)

Plants can respond to stress morphologically, physiologically, and biochemically. Tea plants can adapt to a variety of stresses, but tolerance levels vary by cultivar. A deep understanding of how tea plants respond to various drought stresses is essential for developing adaptation and mitigation strategies. Morphological, physiological, and biochemical analysis methods provide effective tools to study plant responses holistically. Morphological analysis involves visual observations and physical measurements of plant parts such as leaves, stems, and roots. Physiological analysis involves the study of vital plant functions, including the processes of photosynthesis, respiration, and transpiration. Meanwhile, biochemical analysis focuses on the identification and quantification of important compounds in the plant that play a role in adaptation and defense mechanisms. Therefore, the objective of this review is to critically analyze current methods used to detect drought stress in tea plants across morphological, physiological, and biochemical domains, highlight their advantages and limitations, and propose an integrated framework for more accurate and early detection.

Materials and Methods

The reference search of this review was conducted through Google Scholar and academic websites such as Scopus, Ebsco, and Clarivate using the keywords "drought stress," "*Camellia sinensis*," "climate change," and "morphological, biochemical adaptation". The articles obtained are relevant for further analysis using descriptive methods. Articles published between 2007 and 2024 were considered to ensure the inclusion of recent and appropriate findings. A total of approximately 54 articles were initially retrieved. Inclusion criteria comprised original research articles, review papers, and book chapters that addressed drought stress detection in tea plants through morphological, physiological, or biochemical approaches. Studies focusing on general drought stress in other crops were included only if they provided comparative insights applicable to tea plants.

Results and Discussion

Morphological Responses of Tea Plants under Drought Stress

Drought stress reduces plant height, decreases the number and area of leaves, reduces the fresh and dry weights of the leaves, significantly reduces root length and weight, and reduces the content of leaf photosynthetic pigments (Shil & Dewanjee, 2022) Tea plants exhibit various morphological adaptations to cope with drought stress, which are crucial for maintaining their growth and productivity. These adaptations include changes in leaf structure, root development, and overall plant architecture.

Leaf Morphology and Anatomy. Under drought stress, tea plants exhibit smaller leaves, thicker cuticles, and reduced stomatal conductance to minimize water loss. In tea plants, which are cultivated in tropical and subtropical regions with seasonal rainfall, the ability to maintain high water content during drought periods is an important physiological feature in abiotic stress resistance. The cuticle layer becomes thicker, enhancing the leaf's ability to retain moisture. Additionally, there is an increase in trichome (leaf hair) density, which reflects sunlight and reduces leaf temperature, further conserving water. Stomatal density may also decrease, leading to reduced transpiration rates. These modifications collectively enhance the plant's drought tolerance (Qian et al., 2018).

Relative water content (RWC) decreases significantly during drought but recovers partially upon rehydration (Shen et al., 2022). RWC is a physiological parameter that reflects the water status of plant tissues, particularly their hydration level relative to their maximum water-holding capacity. Relative water content is a direct measure of the dehydration state of plant organs and is tightly linked with cell volume and turgor under most biologically relevant circumstances, and these are likely to be the key drought stress signals to which most plant biochemical systems respond (Sack et al., 2018).

Root System Adaptations. Drought stress is a major environmental constraint that limits water availability for tea plants. One of the key adaptive responses to this stress is modification of root system architecture (RSA)—particularly an increase in root depth, length, and biomass to improve water acquisition from deeper soil layers. Drought is one of these stresses (Gupta et al., 2020) (Gupta et al., 2020), and roots have evolutionarily to become the first organ that senses the changes in soil moisture and adapts to them at morphological, anatomical, and molecular scales (Zahedi et al., 2024) (Amtmann et al., 2022)(). Fresh water availability is projected to decline by 50% owing to climate change, whereas water demand for agriculture is expected to double by 2050 (Gupta et al., 2020)Temperature extremes, whether too hot or too cold, can damage plant tissues, disrupt physiological processes, and reduce the quality and quantity of tea production. In addition to the changes already mentioned, tea plants may also undergo modifications to the root system in response to environmental stress. For example, plants may develop deeper roots or increase the production of lateral roots to enhance the uptake of water and nutrients from the soil, which may become more difficult to access due to unfavorable environmental conditions. Tea plants subjected to drought stress often develop deeper and more extensive root systems. This adaptation allows them to access water from deeper soil layers, improving their ability to withstand prolonged dry periods.

Drought rhizogenesis, where new roots form under drought conditions, has been observed in some plant species, enabling rapid water uptake upon rehydration (Thiep et al., 2015). Root system architecture (RSA) is the spatial distribution of roots in the soil profile (Pandey & Bennett, 2019)and it is primarily shaped by length, branching, angle, and thickness. Root System Architecture is characterized by a series of traits including rooting depth, root growth angle, root-to-shoot ratio, root diameter, root length density, root surface area, root volume, root distribution, root tip frequency, and root hair development (Germon et al., 2020). Roots have developed the ability to change the RSA traits in response to water stress ('plasticity') (Kang et al., 2022). A review showed that drought decreased total root length and tip frequency, increased rooting depth, and had no effect on root branching in tree species (Brunner et al., 2015). According to Xiao et al., (2020) the root system configurations, such as root hair, root branches, and root density, can significantly affect the water deficiency of plants.According (Zhou et al., 2018) found that tea seedlings exposed to progressive drought for 21 days had a 46% increase in root mass fraction and a significant increase in specific root length (SRL), allowing the plant to exploit a larger soil volume for water absorption. Furthermore, according to (Wang et al., 2020) that root scanning and 3D modeling demonstrated that tea plants under drought conditions increase vertical root penetration by an average of 12.4 cm deeper compared to control plants. The study emphasized that drought-stressed plants showed a shift toward fewer but thicker roots in upper layers and denser root networks in deeper soil strata (>30 cm depth).

Stem and Branch Modifications. Drought stress can lead to reduced stem elongation and altered branching patterns in tea plants (Rokhmah et al., 2022). These changes result in a more compact plant architecture, which reduces the overall water requirement and exposure to sunlight, thereby minimizing water loss. Drought stress can reduce cell division and expansion, nutrient uptake and transport, and alter phytohormone metabolism and signaling, as well as general metabolism in plants (Xiao et al., 2020). Reductions in stem elongation and photosynthetic rates can result from acclimation and can intensify with increasing stress (Xu et al., 1997). The reduction in stem elongation under drought stress may result from a reduction in cell division, expansion, or both. Cell expansion, an increase in cell volume, is very sensitive to drought stress. Reduced cell size is often observed across a range of drought severities (Zhao et al., 2011). Loosening of the cell wall to increase plasticity and the presence of adequate turgor pressure are key factors that facilitate cell expansion (Cosgrove, 2015). Cell wall extensibility is affected by multiple enzymes, including expansins. Expansins increase loosening of the cell wall, potentially by breaking bonds among cellulose microfibrils and/or hemicelluloses, thereby allowing turgor pressure to expand the cell (Cosgrove, 2015)

In addition, drought stress often triggers stomatal closure in tea leaves to reduce water loss, which limits CO_2 intake and inhibits photosynthesis. As a result, the growth rate is reduced, and the formation of new leaves is inhibited. Extreme temperatures, such as heat waves, can cause thermal stress that damages proteins and cell membranes, while low temperatures can freeze plant tissues. All these factors result in reduced yield and quality of tea production. Proper agronomic management, including efficient irrigation, plant protection, and selection of stress-resistant varieties, is essential to reduce the negative impact of environmental stress on tea plants (Samarina et al., 2020).

Physiological Responses of Tea Plants under Drought Stress

Photosynthesis Performance. Environmental stresses such as drought extremes significantly impact tea plants' growth and morphological development. Drought causes water stress that inhibits photosynthesis, reduces leaf growth, and affects water use efficiency. Excess or lack of water can also cause stress conditions, as water is a core environmental factor affecting plant growth and development (Rezamela et al., 2020)

Photosynthesis is the essential metabolic activity that produces energy and carbohydrates for the growth and development of tea plants. Drought reduces photosynthesis due to stomatal closure, limiting CO_2 uptake (Ding & Jiang, 2022). This leads to decreased carbohydrate synthesis and overall energy production (Gu et al., 2020). The foliar photosynthetic rate is decreased with the decrease of relative water content (RWC) and water potential under drought stress (Qian et al., 2018)

Tea plants regulate their growth and development under temperature stress through physiological and biochemical adaptations, such as adjusting their photosynthetic machinery, activating stress-responsive genes, and accumulating compatible solutes to maintain cellular osmotic balance. Drought stress reduces the availability of water for tea plants, leading to stomatal closure, decreased photosynthesis, and ultimately, reduced growth. Tea plants respond to drought stress by closing stomata to minimize water loss, developing a deeper root system to access deeper soil moisture, and synthesizing protective compounds like proline and antioxidants to mitigate oxidative damage caused by drought-induced reactive oxygen species (Morris, 2023)

When exposed to drought stress, physiological changes in tea plants include various complex responses that affect overall plant growth and development. In addition, drought stress can affect the photosynthesis process of tea plants. Decreased photosynthesis can occur due to disruption in the carbon dioxide uptake process or damage to photosynthetic pigments due to oxidative stress. Plant cells may also change the cell membrane's structure and composition, affecting the cell's permeability to water and nutrients. As a result, the tea plant may experience a decrease in its uptake of water or essential nutrients from the soil, which may hinder its growth and development (Maritim et al., 2015)

Prolonged drought stress can also lead to chronic stress conditions that inhibit the overall growth and productivity of the tea plant. One of the main changes occurs in the chlorophyll content, a pigment that plays an important role in photosynthesis. Drought stress and extreme temperatures often lead to a decrease in chlorophyll content, which impacts photosynthetic efficiency and plant growth (Li et al., 2024).

Nutrient Use Efficiency. Nutrient use efficiency (NUE) refers to the ability of plants to absorb, translocate, and utilize nutrients efficiently for growth, development, and yield production. Under drought stress, NUE become a critical factor as water scarcity directly affects nutrient availability, uptake, and metabolism in plants. This is particularly important in tea plants which are often grown in regions prone to periodic drought. Mineral nutrients play electrochemical, structural, and catalytic roles in all biological organisms and are essential for the completion of the plant life cycle (Lopez et al., 2023). Abiotic stresses and nutrient deficiency severely impact the growth, development, and productivity of plants (Shikha et al., 2023). Environmental changes cause abiotic stress in plants primarily by altering the uptake and utilization of the nutrients. Maintaining nutrient use efficiency under abiotic stress is an effective means of increasing plant stress resistance. Thus, the intensification of abiotic stresses will require the development of plants with high nutrient use efficiency (Liang et al., 2023). For tea plants, maintaining NUE under drought stress is vital for sustaining yield and quality. Strategies to enhance NUE include:

Breeding for Drought-Resilient Varieties: Developing tea cultivars with traits such as deeper root systems or higher water use efficiency (WUE) can improve Nutrient Use Efficiency under drought conditions. WUE is an important parameter in assessing how efficiently plants use water to produce biomass or photosynthetic products. Under drought stress conditions, WUE is very relevant because it reflects the ability of plants to adapt to water limitation to improve the production of adapted varieties, future breeding programs must combine desirable plant traits that complement climate, soil, and management practices (e.g., sowing dates, fertilization, plant density, etc.) in target production systems (Varshney et al., 2021).

Optimized Fertilization Practices:

Fertilization optimization is one of the important strategies in managing drought stress in tea plants especially in the context of climate change, which increases the frequency and intensity of droughts in plantation areas. Tea plants are very sensitive to water availability, and a lack of water can inhibit physiological processes such as photosynthesis, nutrient uptake, and the formation of secondary metabolites that determine yield quality. Therefore, proper application of fertilizers in terms of type, dosage, timing, and application method can help improve water use efficiency (WUE), stimulate root growth, and strengthen the plant's defense system against drought stress (Zerfu, 2018). Fertilizer management enhances WUE by promoting stomatal regulation, osmotic adjustment, and the maintenance of leaf turgor pressure under water-limited conditions (Farooq et al., 2009). Adequate nitrogen and potassium supply, for example, supports deeper and more extensive root development, improving water and nutrient acquisition from the soil (Xu et al., 2010). Moreover, balanced fertilization can upregulate the synthesis of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which protect plant cells against oxidative damage caused by drought-induced reactive oxygen species (ROS) (Ashraf, 2010).

Nutrients such as nitrogen (N), phosphorus (P), and potassium (K) play a crucial role in enhancing the drought resistance of tea plants. Nitrogen supports the synthesis of proteins and chlorophyll that are important in maintaining photosynthetic activity, while phosphorus strengthens root growth, thereby increasing the plant’s ability to absorb water. Potassium has a special role in regulating stomatal aperture and maintaining osmotic balance, which is important in maintaining cell turgor in times of water deficit (Taiz and Zeiger, 2015). The nutrient supply is crucial to improving tea plant health and productivity, Fertilizer optimization is a key strategy to manage drought stress in tea by improving water use efficiency, enhancing root development, and supporting physiological resilience (Que & Zhao, 2024). Balanced application of nitrogen, phosphorus, and especially potassium helps maintain photosynthesis, regulate stomatal function, and promote osmotic adjustment under water deficit. The increased uptake of nutrients from soil due to the application of chemical nutrients or biofertilizers might have produced enough carbohydrate in leaves for translocation to the sink for maximum productivity (Easwaran et al., 2023). Biofertilizers such as mycorrhizae and phosphate-solubilizing bacteria can enhance nutrient uptake and root health, increasing the plant’s ability to access moisture during drought. Integrating organic and inorganic fertilizers improves drought tolerance and tea yield by supporting antioxidant defense and metabolic stability. The integration of organic and inorganic fertilizers enhances drought tolerance in tea by improving soil structure and water-holding capacity (via organic inputs), while ensuring immediate nutrient availability (via inorganic sources). This synergy promotes root development, osmotic adjustment (e.g., proline and soluble sugars), and activates antioxidant enzymes, thereby stabilizing cellular metabolism under drought stress. The improved nutrient balance also supports chlorophyll maintenance and secondary metabolite production, contributing to sustained yield and quality under water-limited conditions (Manzoor et al., 2024). Thus, targeted fertilization not only mitigates drought impacts but also sustains tea productivity under climate stress (Easwaran et al., 2023). Integrated fertilization improved the soil nutrient status, which is associated with the improvement of tea growth and quality (Manzoor et al., 2024)

Soil Moisture Conservation Techniques: The scarcity of water caused by rising temperatures and unpredictable rainfall patterns is responsible for the limited crop yields in arid and semi-arid regions (Li et al., 2018). Hence, it is crucial to manage water usage on farmland to preserve water resources in agricultural areas. Implementing mulching or other soil moisture retention practices can mitigate the effects of drought, thereby supporting consistent nutrient availability and uptake (Demo & Bogale, 2024). Mulching helps conserve water resources, reduce soil erosion, and minimize nutrient runoff, which can improve water quality (Blaise et al., 2021). Among the reviewed strategies, optimized fertilization practices emerge as one of the most directly effective methods to enhance NUE under drought stress. The reason is that targeted nutrient management immediately addresses the nutrient imbalance caused by limited water availability. Overall, a combination of optimized fertilization practices with soil moisture conservation offers a practical and immediately implementable solution, while breeding for drought-resilient varieties is crucial for long-term resilience. The following table summarizes the comparison of NUE strategies under drought stress:

Table 1. Summarizes the comparison of nutrient use efficiency (NUE) strategies under drought stress

Strategy	Mechanism	Strength
Breeding for drought-resilient varieties	Develops traits like deep roots and higher WUE	Sustainable long-term solution, genetic improvement
Optimized fertilization practices	Enhances nutrient availability and uptake	Immediate effect: improves physiological resilience
integrated fertilization (chemical + biofertilizers)	Enhances nutrient absorption and soil health	Improves drought tolerance, boosts antioxidant systems
Soil moisture conservation techniques (e.g., mulching)	Retains soil water, supports consistent nutrient uptake	Easy to implement, cost-effective

Biochemical Responses of Tea Plants to Drought Stress

Tea plants exhibit various biochemical responses to mitigate the adverse effects of drought stress. These responses are crucial for maintaining cellular homeostasis and ensuring plant survival under water-deficient conditions.

Phytohormone. Plants accumulate hormones [e.g., abscisic acid (ABA) or salicylic acid (SA), or methyl jasmonate (MJ) under abiotic stress conditions and pathogen attack. Among the hormones, Abcisic acid is involved in several abiotic and biotic stress conditions and is therefore considered an essential and versatile compound. In contrast, SA, MJ, and ethylene (ET) help in resistance against biotic stress (Bharath et al., 2021). Abscisic acid (ABA) is a stress hormone that accumulates under different abiotic and biotic stresses. A typical effect of ABA on leaves is to reduce transpirational water loss by closing stomata and simultaneously defending against microbes by restricting their entry through stomatal pores (Bharath et al., 2021). When plants were exposed to water stress (drought), an increase in ABA was typical due to either synthesis or degradation of ABA or both (Chen et al., 2020)(Ma et al., 2018)(; (Gietler et al., 2020)). The soil-water deficit could be perceived as a signal by roots to trigger ABA's de novo synthesis (Qi et al., 2018). Salicylic acid (SA) is an endogenous growth regulator naturally produced by plants and belongs to the group of phenolic acids. It plays an important role in regulating plant growth, development, and physiological processes such as photosynthesis and metabolism. SA also enhances plant resistance to biotic and abiotic stresses (Damayanthi et al., 2023).

Under drought-stress conditions, ABA and Salicylic Acid (SA) contents rapidly accumulated in the cells of tea plants, and drought-resistance tea cultivars showed higher ABA and SA contents than drought-susceptible tea cultivars (Liu et al., 2015). ABA is generally emphasized and regarded as a key drought-sense signal from root to shoot. Stomatal closure can also result from direct evaporation of water from guard cells through an active metabolic process involving the reversal of ion fluxes responsible for stomatal opening. This process is regulated by ABA, which mediates signaling between shoots and roots (Sharma et al., 2023). ABA limits transpiration water loss and gas exchange by inducing stomatal closure (Kishor et al., 2022).

Secondary Plant Metabolites. Drought negatively impacts the quality of active ingredients in medicinal and aromatic plants (Emami et al., 2024). Water availability is a crucial environmental factor influencing the growth, development, and synthesis of medicinal plant constituents. Drought stress can influence the biosynthesis of secondary metabolites in tea plants, such as flavonoids and tannins. These compounds have antioxidant properties and may play roles in mitigating oxidative stress. Changes in secondary metabolite profiles can also affect the quality attributes of tea, including flavor and aroma. Water stress causes a reduction in plant size, reduces plant leaf area, decreases whole biomass, and not only alters the plant structurally and anatomically but also leads to fluctuation of their secondary chemical constituents. Secondary plant metabolites (SPMs) are useful to assess the quality and quantity of the therapeutic ingredients, and such metabolites synthesized by the plant help to cope with the negative effects of stress for adaptation and defence (Shil & Dewanjee, 2022).

On the other hand, under drought conditions, tea plants can synthesize a wide variety of flavonoids, including anthocyanins, flavonones, flavonols, and flavanols. As important secondary metabolites in tea plants, flavonoids not only play a role in the stress response of tea plants but also have a significant impact on tea quality (Dong et al., 2019). Flavonoids are responsible for the bitter taste of tea infusions by activating bitter receptors in humans. Flavonoid glycosides are also the main pigments in green tea infusions that determine the appearance and quality of green tea (Li et al., 2023). These secondary metabolites not only serve as defense agents against pathogens and pests but also have important roles as antioxidants that help reduce drought damage. Under the influence of environmental stresses such as drought and temperature extremes, the biochemistry of tea plants undergoes significant changes that reflect adaptation and stress response. One common response is the increased production of antioxidant compounds such as polyphenols and flavonoids. Tea plants produce these compounds as a defense mechanism against environmental stress, such as excessive UV exposure or extreme temperatures.

Proline. Proline degradation is equally essential for supplying energy under long-term stress and when the plants are relieved from stress. Proline is synthesized in chloroplasts/cytoplasm but transported through proline porters to the root and shoot tips, where it supplies energy by oxidation in mitochondria (Kishor et al., 2022). In addition, under environmental stress conditions, such as drought or excess water, tea plants can show an increase in the production of osmotic compounds such as proline, which help maintain osmotic pressure and water balance in their cells.

Drought spur the formation of proline. Proline is one of several small molecules classified as osmolytes or osmoprotectants (X. Liang et al., 2013). Proline is one of the amino acids produced by plants when experiencing abiotic stress. One of the abiotic stresses in question is drought stress. Plants that experience drought stress will usually try to make physiological adaptations. One form of physiological adaptation is to produce proline.

Proline is synthesized as a compound that helps the plant maintain cell turgor. Proline content in drought-tolerant plants is seen to increase in accumulation compared to drought-sensitive plants (Masheva et al., 2022). Proline levels can be used as an indicator of drought resistance (Khan et al., 2025), but not all plants produce it, including even drought-resistant species. The following illustrates the potential function of proline and proline metabolism in stress protection (Fig. 1).

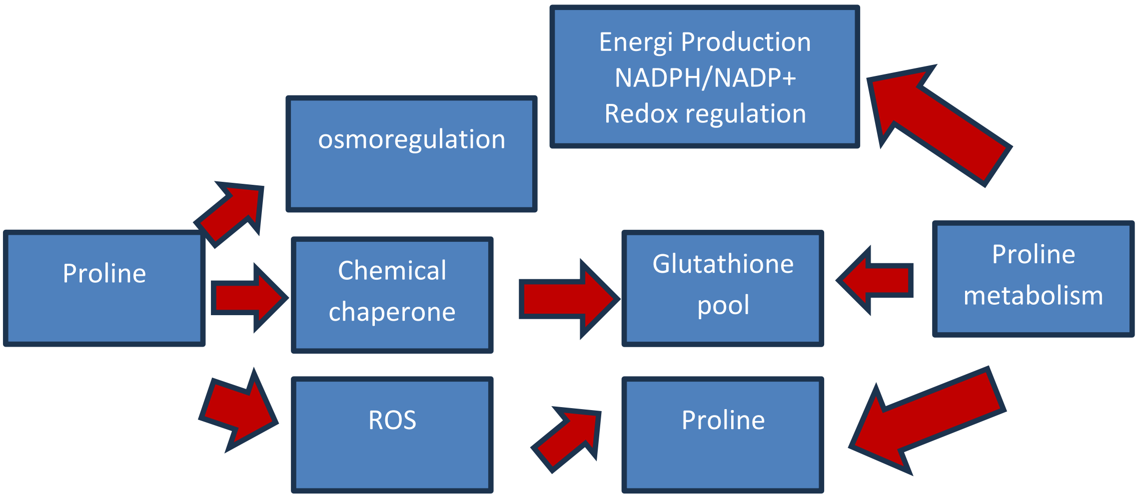

Figure 1. Potential functions of proline and proline metabolism in stress protection (Liang et al., 2013)

The figure shows changes in proline and total sugar levels in tea clones, along with the duration of drought stress. The increase in proline content is a characteristic of plants in acclimatizing to stress. Proline accumulation was highest in clone 276 (50.13%) after 40 days of drought stress, while clone 278 (1.54%) showed the smallest increase compared to control plants. Proline acts as an osmoprotectant during drought stress; high proline accumulation in clone 276 indicates that this clone has genotypic tolerance to drought. Proline accumulation helps maintain water balance and prevent membrane distortion. Under water stress conditions, resistant and tolerant cultivars accumulate higher proline levels than susceptible cultivars (Masheva et al., 2022). Total sugar content increased after 40 days of drought stress by 52.20% and 52.08% for clones 285 and 399, respectively, compared to the control (Chaeikar et al., 2020). During drought stress, especially severe or prolonged, plants utilize soluble sugars optimally to maintain growth.

An increase in total sugar content under drought conditions is a positive characteristic of drought-tolerant plants. Activation of the ROS system helps protect proline and soluble sugars against oxidant stress. Abiotic stress leads to increased accumulation of proline, which acts as an osmolyte. Proline helps stabilize antioxidant proteins and enzymes, scavenge ROS, restore intracellular redox balance, and trigger cellular signaling (M. J. Zhang et al., 2020). Environmental stress also affects enzyme activity and secondary metabolite production. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) have increased activity in response to stress-induced oxidative stress (X. Zhang et al., 2023). Reactive oxygen compounds (ROS) produced in response to stress can damage cells, so an increase in antioxidant enzyme activity helps protect plant cells from oxidative damage (Han & Wang, 2023).

Conclusion

Detection of drought stress in tea plants through morphological, physiological, and biochemical approaches provides a comprehensive picture of plant responses to water deficit. Changes in leaf structure, decreased photosynthetic activity, and accumulation of proline, ABA, and secondary metabolites are important indicators that can be utilized. NUE also plays an important role in strengthening the plant's adaptive response to drought. The integration of these three approaches allows for more accurate identification of stress and supports the development of adaptation strategies to enhance tea plant resilience under climate change. Future research should focus on field experiments evaluating the combined effects of optimized fertilization, biofertilizer application, and soil moisture conservation techniques across different environments would provide practical insights. Exploring the role of microbial inoculants and biostimulants in enhancing physiological and biochemical responses under drought conditions is also recommended. Developing predictive models that link morphological, physiological, biochemical, and environmental data will further support precision management strategies in tea cultivation under climate change.

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Antioxidant activity (2-2-diphenil-1-picrilhydrazil radical-scavenging assay) and phytochemicals of *Mimosa invisa* Colla and *Mimosa pigra* L. grown at different terrestrial habitats

Abstract. Giant false sensitive plants (*Mimosa invisa* Colla) and giant sensitive plants (*Mimosa pigra* L.) are invasive plant species in the tropics and native to America. They are widely distributed in different ecosystems and soil types. Apart from being considered woody shrub weeds, these plant species can be used as herbal medicine for their antioxidant activities. An experiment to study the antioxidant activities and phytochemicals of *M. invisa* and *M. pigra* grown at 200 m from the coastline and close to the riverbank (riparian abandoned land) was carried out from March to November 2023. Weed leaves were collected as purposive randomized sampling from different terrestrial habitats in the City of Padang, West Sumatra. Antioxidant activity was identified according to a 2-2-diphenyl-1-picrilhydrazil (DPPH) radical-scavenging assay, and phytochemical compounds were identified qualitatively. Results demonstrate that *M. invisa* had mild antioxidant activity and *M. pigra* had strong antioxidant activity. The IC_{50} values for *M. invisa* grown at 200 m from the coastline and at the riverbank were 121.3 ± 11.5 and 105.6 ± 2.90 ppm, respectively. The IC_{50} values of *M. pigra* grown at 200 m from the coastline and at the riverbank were 80.6 ± 15.9 and 85.1 ± 9.03 ppm, respectively. Phenolic, saponin, and steroid were detected in all weeds grown at different habitats. However, alkaloids and terpenoids were not detected. Interestingly, flavonoid was not detected in the leaves of *M. pigra* grown at the riverbank.

Keywords: Antioxidant · DPPH · Mimosa · Phytochemicals

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Introduction

The presence of weeds in agricultural lands has attracted serious attention and requires precautionary measures. Global agricultural production systems need to ensure feeding the ever-growing population (Bana et al., 2020; Cheriere et al., 2020; Dossou-Yovo and Saito, 2021; Kumar and Jagannath, 2021) , though weeds cannot be avoided. The reduction in crop growth and yield in the presence of weeds may result from resource competition (Dilliott et al., 2022; Landau et al., 2022; Satorre et al., 2020) and/or allelopathy (Mayerová et al., 2018; Sheldon et al., 2021).

Giant false sensitive plants (*Mimosa invisa* Colla) and giant sensitive tree (*Mimosa pigra* L.) are invasive plant species in tropical areas and widely distributed in different ecosystems and soil types. These species have a high diversity (Wan & Wang, 2018). Weeds of the members of Genus Mimosa have adverse effects on crops and animals that eat the leaves or fruits of these plants. Research has demonstrated that *M. pudica*-containing feed resulted in neurodegeneration in mice (Anderson et al., 2023). Another study revealed that *M. tenuiflora* has caused dysfunction in the reproductive system in some animals such as goat, cattle, and sheep. The calves of these animals experienced arthrogryposis, scoliosis, lordosis, and malformation in the rib bones (Riet-Correa et al., 2023).

Despite their negative impact on crops and animals, various weed species, including *Mimosa* spp., have demonstrated potential for medicinal plants for their antioxidant activities. Medicinal plants have been used for centuries in many countries, including Indonesia. *M. pigra* has been used for its antimicrobial activities against skin infections, diarrhea, malaria, tuberculosis, and persistent cough (Chinsembu et al., 2019). Another species, *M. malacophylla,* that is easily found in northern Mexico, has been used by local people as an ethnomedicinal plant for its diuretic effect and to treat kidney diseases (Guillén-Meléndez et al., 2022).

Various studies, mainly in pharmacology, on the antioxidant activities of (medicinal) plants have been reported, including those of *Mimosa* spp. Some secondary metabolite compounds have been isolated and identified from the weeds of the Genus Mimosa. For instance, *M. pudica* leaves have flavonoid-O-glycoside (Hawwal et al., 2021), tannin has been isolated from *M. tenuilora* (Hernandez et al., 2021), phenolics, tannin, phlobatannins, alkaloids, and saponin have been extracted from the leaves of *M. pigra* (Koodkaew et al., 2018). These compounds have been studied for their potential to cure human health problems. Another study has revealed the potential of the nanoflowers of *M. pudica* for the bioremediation of hazardous pollutants from industrial wastewater (Sharma & Basu, 2021). These works open the window of seeing weeds from different perspectives and change weeds' negative impacts into benefits.

The biosynthesis of plant secondary metabolites may be modified by some environmental factors such as soil salinity (Sivasamy et al., 2022), light spectrum (Peng et al., 2024), and drought, with the help of arbuscular mycorrhiza (Qian et al., 2024). Plant nutritional status plays a significant role in this biosynthesis, as well. *M. invisa* and *M. pigra* are found in many areas in the City of Padang, the Province of West Sumatra, Indonesia, especially on the riverbank (riparian abandoned land) and in places not so far from the coastline. The study was aimed at determining the antioxidant activities and phytochemical compounds of *Mimosa pigra* and *Mimosa invisa* grown at different terrestrial habitats, i.e, at 200 m from the coastline and the riverbank.

Materials and Methods

The experiment used a descriptive method and was conducted from March to November 2023 at the Laboratory of Plant Physiology, the Faculty of Agriculture, Universitas Andalas Padang, Indonesia. The leaves of *M. invisa* and *M. pudica* were collected from different habitats in the City of Padang following the purposive sampling method. The leaves were collected from three trees and composited within the same species and habitat. The weeds collected were triplicated. All sampling sites are located in the City of Padang with soil type of Ultisol, with more sand found in the coastal area. The average monthly rainfall during the experiment ranged from 44.80 mm to 624.00 mm, and the highest rainfall was recorded in June, which was 624.00 mm. However, plant samples were collected in April with an average monthly rainfall of 275.50 mm (BPS, 2025).

Preparation and measurement of antioxidant activity. The leaf antioxidant activity was measured following the DPPH free radical-scavenging assay method (Brand-Williams et al., 1995). This method is the most common for analyzing antioxidant activity (Gulcin & Alwasel, 2023). The leaves of the target plants were hot-air dried in an oven at 65°C for 12 h until the leaves were crispy and the water content reached approximately <10%. The dried leaves were then finely ground, called 'simplicial', stored in air-tight containers, and placed at ambient temperature for later use.

Preparation of the leaf extract for determining the phytochemical compounds and antioxidant activity. Leaf extraction was prepared with maceration in a methanol solution. Five g of simplicial was placed into a 125-mL reagent bottle, then 50 mL of methanol solution (60% methanol + water in 1:1 v/v) was added and macerated for 24 h at ambient temperature. The bottle was lightly shaken every 8 h. After 24 h of the maceration process, the solution was filtered through No. 1 Whatman filter paper. The extraction was triplicated, and the aliquots were pooled in one bottle and kept for 2 days at the laboratory to allow some parts of the liquid to solidify and sediment. The aliquot was then dried using a Rotary vacuum Evaporator Buchi® at 50°C to get a dark green and thick extract solution called "leaf extract".

Measuring the antioxidant activity. The antioxidant activity was assessed using a DPPH free radical-scavenging assay with ascorbic acid with minor modification (Brand-Williams et al., 1995). A 0.1 mM of DPPH solution was prepared and kept in a reagent bottle, which was tightly wrapped with aluminum foil to inhibit light interception. The leaf extract solution was prepared at 100, 200, 300, 400, and 500 ppm. Each concentration of the extract solution was taken for 0.2 mL and mixed with 3.8 mL of 0.1 mM DPPH solution before being thoroughly mixed in a vortex. The mixture was then incubated in the dark at ambient temperature for 30 min. The reduction in the absorption of DPPH solution following the exposure to an antioxidant was measured in a UV-VIS Spectrophotometer at 517 nm wavelength. A 4 mL of DPPH was used as the control sample. The ascorbic acid solution was used as a positive control. The measurement of each sample was carried out three times. The 50% DPPH inhibitory concentration (IC_{50}) was calculated with linear regression. The IC_{50} value is the leaf extract concentration necessary to inhibit 50% of DPPH free radicals and is determined through the linear regression equation formula. The percentage of inhibition was estimated using the following equation:

$$\text{inhibition (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \text{ x } 100$$

Assessing the phytochemical compounds (Harborne, 1998)

The presence of phytochemical compounds in the leaf extract were determined qualitatively.

Flavonoid. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then 0.05 mg of Magnesium and 1 mL of concentrated HCl were added. The mixture was then thoroughly mixed in a vortex until homogeneous. The appearance of red-orange color indicates the presence of flavonoids.

Phenolic. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with ten drops of 10% $FeCl_3$, followed by homogenisation with a vortex for 15 seconds. The appearance of a greenish-blue color indicates the presence of phenolic compounds.

Alkaloid. Forty mg of M. *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with ten drops of concentrated H_2SO_4 followed by thorough mixing. The mixture was then added with five drops of Meyer's reagent. The formation of white precipitation indicates the presence of alkaloids.

Terpenoid and steroid. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then 0.05 mL acetic acid anhydride and 2 mL of concentrated H_2SO_4 were added. The mixture was then thoroughly mixed in a vortex for 15 seconds and then settled for 1 minute. When green, blue, or purple are formed, the extract contains steroids. A similar procedure was repeated, and the presence of red or brown color confirmed the presence of terpenoids.

Saponin. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with 1 mL of distilled water, and two drops of concentrated HCl. The mixture was then vertically shaken for approximately 30 seconds. The extract contains saponin when 1-10 cm height air bubbles were formed and stable for 10 minutes.

Data analysis and presentation. The data on antioxidant activity of each *Mimosa* sp grown at different terrestrial habitats were calculated for the mean values ± SD and are presented in a table. The presence or absence of phytochemical compounds is demonstrated in another table.

Results and Discussion

The antioxidant activity of *M. invisa* and *M. pigra* grown in different terrestrial habitats is demonstrated in Table 1. *M. invisa* grown at 200 m from the coastline and riverbank had mild antioxidant activity with IC_{50} values of 121.3 and 105.6 ppm, respectively. In contrast, antioxidant activity was found to be strong in *M. pigra*, with the corresponding IC_{50} values of *M. pigra* being 80.6 and 85.1 ppm, respectively.

Table 1. Antioxidant activity of *Mimosa invisa* and *M. pigra* grown at two terrestrial habitats

Species	Habitat	%inhibition (IC_{50}) (ppm)
Mimosa invisa	200 m from coastline	121.3 ± 11.5
	riverbank	105.6 ± 2.90
Mimosa pigra	200 m from coastline	80.6 ± 15.9
	riverbank	85.1 ± 9.03

Note: Data are presented as mean values ± SD

Plants belonging to the genus Mimosa are known for their ethno-herbal medicine to cure asthma, bronchitis, and fever (Bezerra et al., 2023). Different terrestrial habitats may result in various growth and physiological status of the plants. The plants grown near the coastline may be exposed to different soil nutrient content compared to other habitats. Areas close to the coastline may contain high salinity that affects the growth and production of plants. Salinity stress in soil may cause damage to root systems. Salt stress may also lead to an imbalance in the osmotic pressure, causing a variety of physiological changes (Shabbir et al., 2023), including photosynthetic and respiration rates (Habibi et al., 2021), leading to disturbance in primary or secondary metabolisms in plants.

Various plants have been used as herbal medicine for their free scavenging and antioxidant properties in countries such as *Euphrasiae stricta*, *Euphorbia platyphyllos* L., and *Epimedium brevicomum* Maxim. in Suadia Arabia for their phenolic and flavonoid content (Jafri et al., 2023), *Mimosa pudica* L. in Malaysia for its flavonoid and phenolic content (Baharuddin et al., 2021), and *Mimosa acutistipula* leaves in Brazil for the presence of tannins, flavonoids, phenol, alkaloids, and terpenes (Bezerra et al., 2023). Our finding is in accordance with previous research that *Mimosa* sp. produced various secondary metabolites, including flavonoids, phenolics, steroids, and saponin.

A phytochemical analysis of *M. invisa* and *M. pigra* leaves revealed that alkaloids and terpenoids were not detected in the samples (Table 2). The absence or presence of certain phytochemicals from various plant species is obvious and is affected by biotic such as soil microorganisms (Singh et al., 2021) and/or abiotic factors, such as seasonal temperatures (Mall et al., 2019). Interestingly, alkaloids and terpenoids were absent from the leaf extracts of both Mimosa species grown in different habitats. It is obvious that the synthesis of secondary metabolites in plants is regulated by certain gene(s). Flavonoids, for example, are major pigments that color most flowers, fruits, and seeds. Research in various plant species such as maize, petunia, and snapdragon has been the major experimental model in understanding the flavonoid-regulating gene(s) (Ferreyra et al., 2012). However, little is known about the regulatory genes for major phytochemicals in Mimosa.

Table 2. The Presence of phytochemical compounds of *Mimosa invisa* and *M. pigra* grown at two terrestrial habitats

Species	Habitat	Fla	Phe	Alk	Ter	Ste	Sap
Mimosa invisa	200 m from coastline	+	+	-	-	+	+
	riverbank	+	+	-	-	+	+
Mimosa pigra	200 m from coastline	+	+	-	-	+	+
	riverbank	-	+	-	-	+	+

Note: + : compound detected, - : compound not detected
Fla = Flavonoids, Phe = Phenolics, Alk = Alkaloids, Ter = Terpenoid, Ste = Steroid, Sap = Saponin

The sampling site of this experiment has different soil characteristics, with more sand found in Ultisol from the coastline than the riverbank. Different soil structures result in different responses in plants' production of phytochemicals. In Al-Jubail, Saudi Arabia, researchers found that soil texture affected plant phytochemical contents, including phenols and flavonoids, and most of the soil structures were loamy sand. They reported that different phytochemical content is related to the degree of genetic similarity (Alotaibi and Abd-Elgawad, 2023). Results of our study have also revealed the presence and absence of flavonoids in *Mimosa pigra* grown 200 m from the coastline and the riverbank, respectively.

Flavonoids, a class of natural products, have been applied for a wide range of purposes, including nutrition, pharmacy, and agrochemicals, including pesticides. These flavonoid compounds have the potential to substitute synthetic chemicals for more environmentally friendly agriculture (Schnarr et al., 2024). The absence of flavonoids from the leaf extract of *M. pigra* grown at the riverbank may be result from the soil condition and other climatic factors. A recent study demonstrated that the concentration of flavonoids in *Phlomoides rotate* increased with altitude in the Tibet Plateau (Li et al., 2024). This finding has confirmed that climatic factors play role in the biosynthesis of secondary metabolites such as flavonoids. The flavonoid of citrus has the potential for biocontrol of pathogenic fungi causing cucumber wilt in seedlings (Wang et al., 2024). Through producing a secondary metabolite, this self-defense mechanism would help nature reduce its dependence on synthetic pesticides.

Phenolic, steroid, and saponin compounds from the leaf extracts were detected in both *Mimosa* sp. grown at different habitats with different soil nutrient content. Another study demonstrated an increase in total leaf phenolic content, flavonoid content, and antioxidant properties of *Echinacea purpurea* L. grown in salt-stress conditions (Ahmadi et al., 2022). The presence of saponin in our experiment is in accordance with another research that reported that saponin content has been reported to increase with low nitrogen supply in *Panax notoginseng* (Cun et al., 2024).

Terpenoids and alkaloids were not detected from the leaf extract in this experiment. The biosynthesis of terpenoid receives carbon supply from the mevalonic acid (MVA) pathway, and the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway, and the phenylalanine-derived phenylpropanoids are a large class (Bergman et al., 2024). Phenylalanine is one of the essential amino acids produced and utilise by plants in biochemical metabolism. Plants need nitrogen to biosynthesize the essential amino acids (Cebani et al., 2024), which can be used for the biosynthesis of secondary metabolites such as terpenoids and alkaloids. The absence of alkaloids and terpenoids from this experiment might be due to a lack of nutrient elements in the soil. The soil where the *Mimosa* sp grew was high in sand, with less in water holding capacity and less in mineral element. Various phytochemicals from the leaf extracts of *Mimosa* sp. correlate with the plants' antioxidant properties. This phenomenon has been reported in quinoa (*Chenopodium quinoa* Willd.) seeds to a certain degree (Yang et al., 2024).

Conclusion

Mimosa invisa and *Mimosa pigra* grown at different terrestrial habitats demonstrated antioxidant activity. *M pigra* had more potent antioxidant activity compared to that of *M. invisa*. Phytochemicals of flavonoids, phenolics, steroid, and saponin were present in both *Mimosa* species. However, alkaloids and terpenoids were not detected in both *Mimosa* species in both habitats.

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Heterologous expression of maize-derived antimicrobial peptide ZmES4 in *Escherichia coli* for potential plant pathogen control

Abstract. ZmES4 is a plant-derived antimicrobial peptide (AMP) from maize that shows promise as a biocontrol agent against plant pathogenic organisms. In the context of growing challenges in sustainable agriculture, AMPs like ZmES4 represent innovative alternatives to chemical pesticides. This study focuses on the structural characterization and heterologous expression of the ZmES4 peptide in *Escherichia coli* (*E. coli*). The gene encoding ZmES4 was obtained from the maize female gametophyte (NCBI Reference Sequence: NM_001112150.3) and cloned into the pET24d(+) expression vector using NcoI and XhoI restriction sites. Transformation into *E. coli* BL21 (DE3) cells enabled recombinant expression upon induction with isopropyl β-D-1-thiogalactopyranoside (IPTG). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Bradford assays confirmed the expression of ORF-ZmES4, with protein concentrations ranging from 14.647 to 63.606 mg/mL. The successful expression of ZmES4 in *E. coli* highlights its potential application as a recombinant AMP for future plant disease management strategies.

Keywords: Recombinant protein production · Defensin-like peptides · Biological control agent · Crop protection

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Introduction

The current challenges in agricultural cultivation have become increasingly complex due to the intensification of both biotic and abiotic stresses. Biotic stresses, such as pests and plant diseases, can significantly reduce crop yields, while abiotic stresses, including drought, excessive rainfall, and fluctuating humidity levels, also negatively impact plant growth and productivity. Climate change exacerbates these stressors, demanding the development of innovative and sustainable solutions to ensure food security (Pandey et al., 2021; Zhu et al., 2020). While the use of resistant crop varieties remains a key strategy in disease management, it is often limited by the emergence of new pathogen races and environmental instability.

An emerging and promising approach to complement existing disease management strategies is the use of antimicrobial peptides (AMPs) derived from plants. In addition to their natural role in plant immunity, certain AMPs like ZmES4 from maize have shown potential when heterologously expressed in microbial systems such as *E. coli* (Bej et al., 2021; Montesinos & Bardají, 2020), enabling their broader application in biocontrol strategies.

Plant-derived AMPs are small, positively charged molecules typically consisting of 20–60 amino acids with amphipathic structures and high cysteine content. These peptides play a crucial role in plant innate immunity by inhibiting the growth of phytopathogenic bacteria, fungi, and oomycetes. Their primary modes of action include disrupting microbial membrane integrity, binding to specific lipid components, or triggering oxidative stress in pathogens (Goyal & Mattoo, 2021). Due to their structural stability and specificity, AMPs have attracted attention as promising candidates for engineering disease-resistant crop varieties across different agricultural sectors, including cereals, vegetables, and industrial crops (Li et al., 2023).

Several AMP genes have been isolated and characterized in the past five years from various plant species such as *Oryza sativa* (rice), *Capsicum annuum* (chili pepper), *Medicago truncatula* (barrel medic), and *Solanum tuberosum* (potato). Each AMP exhibits antimicrobial activity against specific pathogens. For instance, OsDEF7 from rice effectively suppresses *Magnaporthe oryzae*, the causative agent of rice blast, while Snakin-1 from potato shows broad-spectrum activity against both bacterial (*Erwinia*) and fungal (*Fusarium*) pathogens (Singh et al., 2022; Jha et al., 2021). These peptides can be integrated into pest and disease management programs in staple crops, horticultural commodities like chili and tomato, as well as estate crops such as cocoa and oil palm, offering an eco-friendly alternative to chemical pesticides (Yuan et al., 2020; Santos et al., 2023). Ten plant-derived AMPs, their functions, and sources (2019–2024) are shown in Table 1.

The ZmES genes in maize encode peptides that exhibit significant structural homology to plant defensins, a class of small, cysteine-rich proteins known for their antimicrobial functions. Interestingly, these genes are specifically expressed in the cells of the female gametophyte, particularly in the synergid cells adjacent to the egg apparatus, suggesting a specialized role in reproductive processes such as pollen tube guidance or sperm cell release. The tissue-specific expression and structural characteristics of the ZmES peptides highlight their potential function in mediating intercellular communication during fertilization in maize (Cordts et al., 2001). *ZmES4* is one of the member families of these genes.

The potential of ZmES4 and other plant-derived AMPs lies in their integration into crop protection strategies via transgenic expression or bioformulation approaches. As climate change intensifies abiotic stresses such as drought, heat, and irregular rainfall, the vulnerability of crops to disease increases. While the development and deployment of disease-resistant elite varieties remain essential, these strategies alone are not sufficient. There is an urgent need for integrated disease management approaches that combine conventional methods with innovative tools, including the application of AMPs for enhancing plant immunity (Li et al., 2022; Natarajan et al., 2021). These peptides offer a sustainable and environmentally friendly alternative to synthetic pesticides and can play a crucial role in building future crop resilience.

Table 1. Plant-derived antimicrobial peptides (AMPs), their functions, and sources (2019–2024)

No	Gene/ Peptide Name	Plant Source	Target Pathogen(s)	Function/ Mechanism	Reference (Year)
1	OsDEF7	*Oryza sativa*	*Magnaporthe oryzae*	Antifungal; disrupts membrane integrity	Jha et al. (2021)
2	CaAMP1	*Capsicum annuum*	*Colletotrichum gloeosporioides*	Inhibits hyphal growth and spore germination	Li et al. (2023)
3	MtDef4	*Medicago truncatula*	*Fusarium oxysporum*	ROS induction; antifungal	Goyal & Mattoo (2021)
4	RsAFP2	*Raphanus sativus*	*Botrytis cinerea*	Targets fungal glucosylceramides	Yuan et al. (2020)
5	Snakin-1	*Solanum tuberosum*	*Erwinia carotovora*, *Fusarium* spp.	Antimicrobial spectrum broad; membrane disruption	Singh, et al. (2022)
6	NbAMP1	*Nicotiana benthamiana*	*Phytophthora infestans*	Induces systemic resistance	Wang et al. (2023)
7	TcDef	*Theobroma cacao*	*Moniliophthora perniciosa*	Inhibits germination and colonization	Santos et al. (2023)
8	AtPDF1.2	*Arabidopsis thaliana*	*Alternaria brassicicola*, *Botrytis cinerea*	JA/ET-pathway regulated peptide; antifungal	Chen et al. (2020)
9	PaAMP	*Persea americana*	*Pseudomonas syringae*, *Phytophthora cinnamomi*	Broad-spectrum antimicrobial	Morales et al. (2022)
10	EgAMP	*Elaeis guineensis*	*Ganoderma boninense*	Induces apoptosis-like cell death in pathogen	Rahman et al. (2023)

Materials and Methods

Open reading frame (ORF) of ZmES4 gene was extracted from NCBI Reference Sequence: NM_001112150.3 of Zea mays female gametophyte-specific protein ES4 precursor (LOC5426230). ORF fragment (276 base pairs) was inserted between NcoI and XhoI restriction enzyme into plasmid pET24d(+) as vector (Figure 1a and 1b). This plasmid pET-24d(+) was provided by Novagen Specialty Limited, (2024). E. coli BL21 (DE3) (Merck KGaA, Darmstadt, Germany) was used as the expression host. Bacterial strains were cultured in Luria-Bertani medium (supplemented with 50 µg/mL kanamycin when required).

Expression of ORF-*ZmES4 in E. coli* BL21. Following the transformation of *E. coli* BL21 (DE3) competent cells with pET24-ORF-*ZmES4* plasmid by the standard CaCl2 protocol, expression of recombinant ORF-ZmES4 protein was induced by the addition of isopropyl-β-d-thio-galactoside (IPTG) to a final concentration of 1 mM at a bacterial concentration of OD600 = 0.6. Bacterial culture was incubated for 4 h at 37°C in the presence of IPTG on a rotary shaker incubator at 150 rpm. Subsequently, the optical density of bacterial suspensions was measured at 600 nm for each sample right before they were collected by centrifugation. After the washing steps, the pellets were saved at − 70°C for further analyses.

SDS-PAGE analyses. The bacterial pellet was resuspended in an appropriate volume of Laemmli buffer based on their measured OD600 at the time of their collection to normalize the amount of loaded sample (i.e. equalizing their OD measures for the same value) and boiled at 100°C for 10 min and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). To visualize the protein bands, the gel was stained with Coomassie brilliant blue. After image acquisition by a flatbed scanner (Scanjet™ 3800, HP), the yield of expressed recombinant protein was determined by image analysis. To obtain the protein expression yield, the area under the peaks was divided by the total area under the curve.

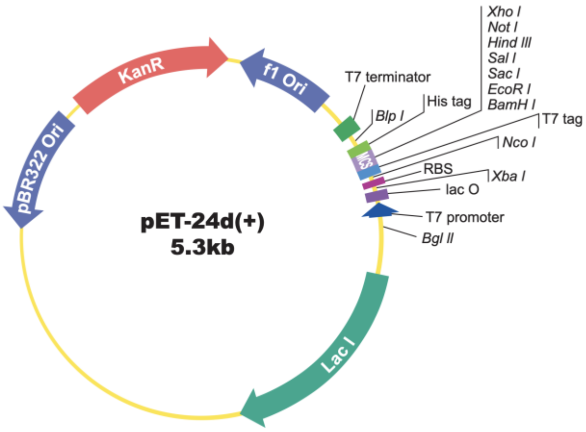

Figure 1a. Plasmid pET24d(+) for inserting ORF of *ZmES4*

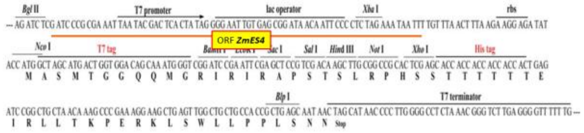

Figure 1b. ORF of *ZmES4* inserted in Plasmid pET24d(+)

Results and Discussion

Plasmid Isolation and Quantification. Three mL of cultures were isolated by Tianprep Rapid Mini Plasmid Kit (alkaline lysis methods). It is shown in Table 2. that Colony 1 and Colony 4 are the best colonies and were sent for Single Pass DNA sequencing. Quantification of plasmid was done through Nanodrop-spectrophotometry methods, at A260 nm. Colony 1 and Colony 4 were determined as the best colonies based on the highest plasmid DNA concentration parameters (202.156 ng/µL and 204.555 ng/µL, respectively, as shown in Table 2). This parameter indicates successful transformation, making them the most suitable candidates for further analysis.

Assessment of ORF-ZmES4 expression by SDS-PAGE. To evaluate the ability of bacterial colonies to express the recombinant ORF-ZmES4 protein, several randomly selected kanamycin-resistant colonies of BL21 (after transformation by pET24-ORF-ZmES4 recombinant vector) were induced for protein expression by the addition of IPTG, and the expression level of these colonies was assessed by SDS-PAGE. The result of SDS-PAGE did not show a visible ZmES4 protein band, likely because the protein is a minor component, as shown in Figure 2. Therefore, purification using Ni-Sepharose was necessary to accumulate the band. Colonies 1 and 4 showed higher expression levels of the ORF-ZmES4 protein compared to the other colonies (Table 2).

Table 2. The concentration of the plasmid containing *ZmES4*

No.	Colony	Concentration (ng/µL)
1	pET24d(+)_ZmES4 Colony 1	202.156
2	pET24d(+)_ZmES4 Colony 2	166.306
3	pET24d(+)_ZmES4 Colony 3	161.749
4	pET24d(+)_ZmES4 Colony 4	204.555
5	pET24d(+)_ZmES4 Colony 5	189.393
6	Control	33.069

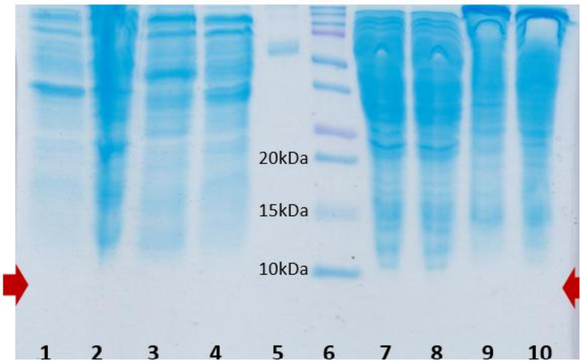

Figure 2. Test Expression Result of ORF-ZmES4 protein

The total protein of ORF-ZmES4 (mg/mL) by Bradford-spectrophotometry methods at A590 nm from ten samples (induced and uninduced) was shown in more detail in Table 3. The protein concentration in the induced sample was lower than that in the uninduced one, likely because the sample contained total protein, not only the target protein.

The maize-derived antimicrobial peptide ZmES4 has gained attention for its role in inhibiting the growth of various plant pathogens, particularly through its cysteine-rich structure that promotes membrane disruption in target microbes. Compared to AMPs from other plants, such as defensins from Arabidopsis thaliana or thionins from wheat, ZmES4 exhibits a unique expression profile in the female gametophyte and has shown effective antifungal activity when heterologously expressed in microbial systems (Bej et al., 2021). While thionins and defensins tend to be broadly expressed across plant tissues and show general antimicrobial activity, ZmES4 appears to have a more targeted expression and may function in a specialized context, offering opportunities for tissue-specific disease management applications (Kim et al., 2022).

Table 3. Total protein of the several samples

No	Sample	Total Protein Concentration (mg/mL)*
1	SF *E. coli* BL21(DE3) Induced	14.647
2	SF *E. coli* BL21(DE3) Uninduced	61.710
3	**SF *E. coli* BL21(DE3)-ZmES4 Induced**	**26.857**
4	SF *E. coli* BL21(DE3)-ZmES4 Uninduced	22.299
5	BSA	-
6	Protein Marker	-
7	IB *E. coli* BL21(DE3) Induced	72.808
8	IB *E. coli* BL21(DE3) Uninduced	27.116
9	**IB *E. coli* BL21(DE3)-ZmES4 Induced**	**63.606**
10	IB *E. coli* BL21(DE3)-ZmES4 Uninduced	70.788

Note: SF = Soluble Fraction, IB = Insoluble Fraction

By implementing the expression of antimicrobial peptides from plants, i.e., maize in *E. coli*, researchers can now exploit new tools for producing recombinant proteins for controlling crop diseases research, and applications. Targeted antimicrobial peptide delivery and viral vector modifications are other new areas of interest that can particularly benefit from this technology (Lesch et al., 2010).

The future potential of ZmES4 and other plant-derived AMPs lies in their integration into crop protection strategies via transgenic expression or bioformulation approaches. For example, recent advances in peptide engineering and delivery systems enable the use of synthetic AMPs with enhanced stability and efficacy under field conditions (Roy et al., 2023). Compared to traditional chemical pesticides, AMPs like ZmES4 are environmentally friendly, biodegradable, and pose a lower risk of resistance development in pathogens. As biotic stressors become increasingly complex due to climate change, AMPs offer a versatile and sustainable tool for the next generation of disease-resistant crops.

Conclusions

1. The ORF-ZmES4 protein was successfully produced using the pET24d(+)-ORF-ZmES4 plasmid construct. The total protein concentration ranged from 14.647 to 63.606 mg/mL after induction.
2. Implementing the expression of antimicrobial peptides from plants, i.e., maize in *E. coli*, is a new tool to produce recombinant proteins for future plant pathogen control.

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Maxiselly Y · Suherman C · Samuel J

The effect of topping and various cytokinin-based plant growth regulators applications on immature Liberica coffee growth

Abstract. The growth of Liberica coffee in the 2-year immature plant phase can be improved by applying cytokinin-based plant growth regulators (PGRs) and topping. Toppings applied to plants can inhibit apical dominance, while cytokinin PGRs such as 6-Benzylaminopurine (BAP) and coconut water can stimulate plant shoot growth. This research aims to determine the effect of topping and PGR application on the vegetative growth of Liberica coffee plants. This experiment was carried out at the Ciparanje experimental field, Universitas Padjadjaran, West Java, with an elevation of ± 750 meters above sea level from March to June 2024. The experimental design used a randomized block design with six treatments: control (no topping and no added PGR), topping, topping + 60 ppm BAP, topping + 50% coconut water, 60 ppm BAP, and 50% coconut water. Each treatment was repeated four times. The results showed the best results were (a) topping treatments (topping, topping+coconut water, and topping+BAP) on canopy width and primary branch length; and (b) a combination of 50% coconut water and 60 ppm BAP on increasing the number of branches. This study provides information on efficient maintenance methods for Liberica coffee plant growth in the immature phase.

Keywords: Benzyl Amino Purine · Coconut water · Cytokinin · Vegetative growth

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Introduction

Coffee is one of the important plantation commodities in Indonesia. Indonesia was ranked the fourth largest coffee producer (Tampubolon et al., 2023). Several coffee species are well-known and produced in Indonesia: Arabica (*Coffea arabica* L.), Robusta (*Coffea canephora)*, and Liberica (*Coffea liberica* L.). Liberica coffee is cultivated on a small scale compared to Arabica and Robusta in the case of Indonesia (Davis et al., 2022; Maxiselly et al., 2023a). It is caused by the Liberica's taste being more bitter than other species, so the consumer has more specific preference (Maxiselly et.al., 2023b). However, Liberica coffee has the potential for resistance to leaf rust disease and to thrive under drought stress (Wibowo et al., 2021; Wahibah et al., 2023).

The Indonesian Bureau of Statistics data from 2021 to 2023 showed a declining trend in national coffee production. In 2023, there was a decrease in coffee production by 27.46 thousand tons compared to the total production in 2021 of 786.19 thousand tons (BPS, 2024). Therefore, maintenance practices need to pursue increasing coffee production.

One crucial on-farm maintenance in coffee production is pruning during the immature phase. Pruning is one of the cultivation methods that contributes to increasing plant production and is one way to harvest plants effectively (NRCS, 2009). Pruning stimulates lateral shoot growth, inhibits apical dominance, and forms an optimal canopy on the coffee plantation (Li et al., 2021; Kawabata et al., 2022). One type of pruning is a topping. Topping is cutting part of the top of primary branches or shoots at a certain height (Hamdani et al., 2021).

Vegetative growth after pruning typically occurs without the need for plant growth regulators (PGRs). However, PGRs may be required to enhance vegetative growth following pruning treatments. PGRs are organic compounds that regulate physiology in small amounts. PGRs include auxin, gibberellin, cytokinin, ethylene, and abscisic acid (ABA) (Fardha, 2024). In this study, cytokinin-based PGRs were used, as this compound promotes lateral shoot growth and stimulates flushing in plants (Purcell, 2017; Sosnowski et al., 2023).

Cytokinin plays a role in cell division that stimulates shoot growth. Cytokinin can be obtained from nature, such as coconut water. The chemical compounds in coconut water include purine, glucose, amino acid, nucleic acid, alcohol, vitamins, minerals, and PGR (Yong et al., 2009; Rosniawaty et al., 2018). Besides cytokinin, the coconut water contained various PGRs, including auxin, ABA, and gibberellin (Emilda, 2020). In addition, synthetic cytokinin can be found in 6-Benzylaminopurine (BAP). BAP is a regulator, stimulant, and inhibitor of plant growth and development (Ramayana et al., 2022).

Coconut water and BAP function by stimulating shoot formation and vegetative growth at certain concentrations. Based on previous research, 50% coconut water has been proven to increase shoot length during the seedling phase in the Robusta coffee plant (Arpansori & Febrialdi, 2020). Furthermore, applying 50% coconut water in the immature phase of tea plants gave an increase in stem diameter, shoot length, number of leaves, and number of shoots (Rosniawaty et al., 2018). Meanwhile, applying BAP 60 ppm has improved shoot growth in tea plants (Ayuningsari et al., 2017; Rosniawaty et al., 2018). Maxiselly et al. (2021) reported that applying 30, 60, and 90 ppm BAP to the cinchona plant significantly responded to increased branch length, number of leaves, and stem diameter.

Several prior studies have reported the combination of pruning treatment and application of PGR. Anjarsari et al. (2021) used pruning techniques with cytokinin and gibberellin on tea plants by applying PGRs after the pruning treatment. De-topping pruning, accompanied by applying 500 ppm and 100 ppm GA, can increase the number of shoots on papaya plants on the 15th and 45th days after treatment (Das et al., 2018). The vegetative growth resulting from PGRs treatment should be compared with pruning treatments, including a combination of both, as no recent study as assessed these treatments on Liberica coffee plants during the immature phase. Based on the explanation above, topping and cytokinin-based PGRs can be combined to increase growth during the 2-year immature plant phase (IPP-2) of Liberica coffee. This study focuses on identifying the impact of topping and/or cytokinin growth regulators on the growth of IPP-2 Liberica coffee. The expected outcome is the discovery of an efficient and applicable method to improve the coffee growth performance in the immature phase.

Maxiselly Y, Suherman C, Samuel J. 2025. The effect of topping and various cytokinin-based plant growth regulators applications on immature Liberica coffee growth. Jurnal Kultivasi, 24 (1): 95-103.

Materials and Methods

This research was conducted from March to June 2024 at the Ciparanje experimental field, Universitas Padjadjaran, West Java, with an elevation of ± 750 meters above sea level. The materials were a 2-year-old Liberica coffee var Meranti 1 (LIM 1), BAP as synthetic PGR, coconut water, and water. The experiment was arranged in a randomized block design (RBD) with six treatment combinations. Each treatment was replicated four times, including control (no topping and no added PGR), topping (T), topping + 50% coconut water (T+CW), topping + 60 ppm BAP (T+BAP), no topping + 50% coconut water (CW), and no topping + 60 ppm BAP (BAP).

Liberica was planted 2 x 2.5 m. Topping was conducted on the plant that had reached a height of 130 cm or more. The technique was to cut the primary branches to a height of 130 cm using pruning shears. PGR formulation was carried out using the procedure: Coconut water was collected at a concentration of 50% (50% of coconut water: 50% of water). Meanwhile, the BAP concentration of 60 ppm is made by mixing 6 ml of BAP into 1000 ml of water. First, the dose to be sprayed is calibrated one day before spraying PGR. BAP and coconut water are applied by spraying them all over the plant canopy using a hand sprayer. The application is performed 0 weeks after treatment (WAT), 2 WAT, 4 WAT, 6 WAT, 8 WAT, and 10 WAT. The time for applied growth regulators is around 8.00-10.00 a.m.

The observed variables included the increase in canopy width (cm), number of branches, stem diameter (mm), branch length (cm), and chlorophyll content index (CCI) (units). The canopy-wide measurement measures the length between the outer branches in opposite directions. The number of branches counted are primary branches. The stem diameter was measured using a caliper at a height of 3 cm from the ground surface. The branch length measured was one primary branch on each plant. A selected primary branch is below the third branch from the top and then marked using a label. The chlorophyll content was measured using a digital chlorophyll meter. The way to use the tool is that the tool was clamped on the leaf of the sample plant until a number appeared on the monitor, and the result is expressed in units of chlorophyll content index (CCI). All observations are identified every 2 weeks from 2 to 12 WAT. The measured observations are then subtracted from the parameter appearance at 0 WAT to obtain the value of the parameter increase.

Data were analyzed using the analysis of variance (ANOVA) with the F test at the 5% level. A Duncan multiple range test (DMRT) was performed at 95% confidence when the variable data had significant differences between treatments.

Results and Discussion

Coefficient variation of observed variables. The data in Table 1-3 and Figure 1-2 showed the result of the observed variables. The observed variables were canopy width, number of primary branches, branch lengths, stem diameter, and chlorophyll content. All of that shows their increasing value. The observed data shows that accuracy starts from CV below 20%. However, various data have found that CV values are more than 20%. There are: increasing canopy wide at 2 weeks after treatments (WAT) and 4 WAT (Table 1); all observed data in Table 2 have CV more than 20%; data of 2, 4, and 6 WAT on increasing branch length (Table 3); and chlorophyll content at 2 WAT and 6 WAT (Figure 2). A CV value of more than 20% indicates high criteria. These data were influenced by external factors, including environmental factors (Utami et al., 2023). Maxiselly et al. (2021) assessed variables in immature coffee plants with a CV of more than 20%, indicating the accuracy of their data.

Increase in canopy-wide. Table 1 shows that the topping and topping with BAP treatments significantly differed from PGRs, with only a group of PGR treatments, such as coconut water and BAP treatments at 6 WAT and 8 WAT. The best treatment to increase the canopy width in those weeks was topping treatments. Although it was not significantly different from the control, it was topped with coconut water and BAP. In 12 WAT, topping and topping with BAP treatments had significant differences from the control, coconut water, and BAP treatments. The best method to increase the canopy width that week is topping with BAP, topping, and topping with coconut water.

The plant is generally composed of two metabolite groups, i.e., primary metabolites and secondary metabolites. Primary metabolites are directly involved in developing plant growth, while secondary metabolites indirectly affect plant growth (Salam et al., 2023). Primary metabolites decode the genetic code that produces amino acids, proteins, and carbohydrates (Julianto, 2019). This can regulate the distribution of primary metabolites by pruning to focus on the desired targeted organ. Prior research by Kumar et al. (2017) stated that the growth of the canopy was affected by pruning due to a shift in plant metabolite allocation to support vegetative growth. Topping pruning can stop apical dominance, which inhibits vertical growth (Obasi & Msaakpa, 2005).

In addition, BAP after topping treatment has a greater increase in canopy width than topping treatment only at 10 and 12 WAT. Although statistically, there is no significant difference between them. Cytokinin treatment after topping can increase the number of lateral buds. The addition of exogenous cytokinins increased the amount of cytokinins in the canopy (Di Benedetto et al., 2015). Anjarsari et al. (2021) reported that the increasing number of lateral buds makes the canopy structure wider on the tea plants.

Increase in the number of primary branches. Table 2 shows that the BAP treatment has better results than other treatments. Meanwhile, topping with BAP had the lowest result in increasing the number of primary branches. Statistically, there was a significant difference between the coconut water and BAP treatments for topping and topping with BAP treatments at 6 WAT and 8 WAT. In 10 WAT, BAP was not significantly different from the control and coconut water treatments. However, the BAP treatment had no significant difference from the coconut water treatment at 12 WAT in the number of primary branches.

The cytokinin content in BAP and coconut water could cause an increase in the number of primary branches. Cytokinin is a plant growth regulator that plays an important role in stimulating the growth of lateral shoots, triggering chloroplast development, and encouraging leaf expansion and cell division in plant tissue (Ramayana et al., 2021).

Table 1. Increase in canopy width from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in canopy wide (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	11.63	16.75	20.00ab	26.00ab	32.25	41.50b
T	11.08	20.38	29.38a	33.88a	39.38	51.375a
T+CW	4.63	17.50	19.75abc	23.50ab	36.50	49.25ab
T+BAP	1.75	13.25	25.00a	33.25a	45.13	56.25a
CW	3.38	3.25	12.50bc	19.50b	32.00	41.00b
BAP	5.25	7.13	10.25c	17.75b	28.75	40.25b
CV (%)	44.06	26.57	15.85	13.89	11.53	17.80

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

Table 2. Increase in the number of primary branches from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in the number of primary branches					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	0.50	0.50	1.25a	1.50ab	1.75ab	1.75bc
T	0.00	0.00	0.00b	0.50bc	0.75bc	0.75bcd
T+CW	0.00	0.00	0.25ab	1.00abc	1.00bc	0.50cd
T+BAP	0.00	0.00	0.00b	0.00c	0.00c	0.00d
CW	0.50	0.50	1.00ab	1.50ab	2.00ab	2.50ab
BAP	0.50	0.50	1.50a	2.50a	2.75a	4.25a
CV	25.24%	25.24%	22.70%	21.12%	21.84%	23.35%

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

As a synthetic PGR, BAP was thought to trigger shoot growth that would become primary branches. Prior research confirmed that applying 60 ppm and 120 ppm BAP positively responded to increasing the number of shoots on tea plants (Saefas et al., 2017). Khalil et al. (2021) stated that BAP treatments amplify the expansions of vascular strands and xylem differentiation to increase the number of branches. As a natural PGR, coconut water contains two types of cytokinin, namely zeatin and kinetin, which accelerate shoot growth (Setyowati et al., 2023). Lazim et al. (2015) stated that the cytokinin content in coconut water could function in cell division, which helps shoot establishment.

Non-topping treatments have caused an increase in the number of branches. The number of branches obtained is greater than the topping treatments. Unpruned plants will produce non-productive branches with larger distances (Takur et al., 2018). The topping treatments did not increase the number of lateral shoots. The pruning, such as topping, will depress apical dominance, elongating the lateral shoot instead of elevating the number of branches (Kebrom, 2017).

Damayanti et al. (2022) stated that the increasing need for sinks influences the photosynthesis rate. A large number of branches and leaves can cause competition for nutrients and the rate of photosynthesis to be less than optimal (Yang et al., 2022). Therefore, a small number of branches after topping can reduce competition between branches and achieve optimal productivity.

Increase in branch length. Table 3 showed insignificant differences among all treatments, from 2 WAT to 8 WAT. Thus, the treatment given to Liberica coffee plants requires time to show its results. Korte et al. (2025) stated perennial plants require treatment time to show the effect that occurs.

The topping treatment differed significantly from that without topping, such as control, coconut water, and BAP treatments, at 10 WAT. The topping and topping+BAP also showed significant differences from control, coconut water, and BAP on 12 WAT. Pruning can influence the growth of branches by switching the allocation of photosynthate in the plant (Dong et al., 2019). The topping pruning treatments made branches grow stronger and longer. Topping can affect assimilation distribution from the apical bud to lateral growth (Hamdani et al., 2021).

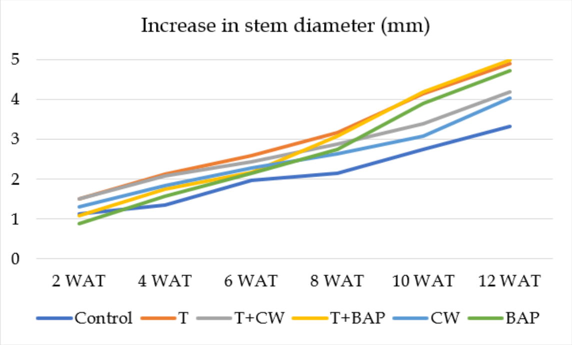
PGR can play a role in stimulating the vegetative growth of plants. BAP and coconut water had cytokinin, which supported vegetative growth (Zanirah et al., 2023). Besides cytokinin, coconut water also contains the hormone auxin. These hormones play a role in the cell division process, which helps with bud formation. Cytokinin promotes cell division, while auxins stimulate cell elongation.

Table 3. Increase in branch length from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in branch length (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	2.50	4.75	6.00	7.50	8.75b	9.75b
T	2.38	5.25	8.38	11.25	14.13a	16.75a
T+CW	1.13	2.50	4.75	9.38	10.75ab	12.63ab
T+BAP	3.30	5.55	6.80	9.80	11.68ab	16.18a
CW	0.25	2.88	4.00	7.38	8.50b	10.50b
BAP	2.13	4.13	5.13	7.50	10.25b	11.63b
CV	31.68%	28.42%	22.62%	12.86%	8.88%	11.11%

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

Increase in stem diameter. Figure 1 shows that all treatments gave insignificant differences in increasing stem diameter. Several previous studies confirmed that pruning treatment has no relation to increased stem diameter. Prior research confirmed that topping did not respond to tomato stem diameter (Nkansah et al., 2021). Gebisa (2023) reported that stem diameter had no response to various pruning treatments on the coffee plant. Apart from environmental factors, genotype factors also influenced the increase in stem diameter parameters. The statement is also supported by Lhamo et al. (2022) that growth is influenced more by cultivars than by pruning treatment. The various pruning methods did not influence the stem diameter in Arabica coffee species, as the diameter develops slowly in woody plants (Wisdawati, 2023). The application of cytokinin, such as BAP and coconut water, influences stem diameter by promoting cell division. Apical dominance leads to competition between the apical and lateral buds during vegetative growth, causing the plant's growth and development to focus more on new shoots and branches (Ayunda et al., 2021).



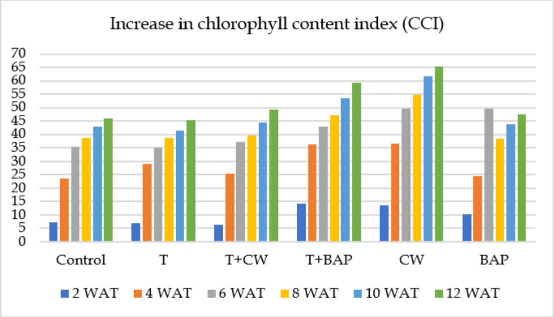
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
CV	15.96%	13.92%	12.76%	14.1%	17.21%	14.89%

Note: WAT=Week after treatment, CV=Coefficient of Variation, T=, CW= 50%Coconut Water, BAP= 60 ppm 6-Benzylaminopurine.

Figure 1. Increase stem diameter from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Increase in chlorophyll contents. Figure 2 shows that the topping and/or application of PGRs had no significant effect. This is because the treatments given are not related to sunlight entering easily. Topping is a technique for manipulating the ideal environmental conditions and supporting photosynthesis. The leaves under the stem that have been pruned act as food reserves produced from the process of photosynthesis (Anjarsari et al., 2021). These food reserves play a role in the growth of new shoots and leaf development. Grati et al. (2016) stated that the pruning that changes the plant's shape to let sunlight in more easily could affect the chlorophyll content.

It has been found that there is an increase in the chlorophyll content index with leaf age. Wanda et al. (2022) stated that leaf age is a significant factor in increasing a leaf's chlorophyll index, apart from sunlight. Metabolites in leaves that are degraded due to senescence are then distributed to other tissues that are still actively growing (Kanojia et al., 2021).



	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
CV	32.99%	19.34%	20.69%	18.28%	16.28%	15.78%

Note: WAT=Week after treatment, CV= Coefficient of variation, T=Topping, CW= 50%Coconut Water, BAP= 60 ppm 6-Benzylaminopurine.

Figure 2. Increase in chlorophyll content from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Conclusion

The topping and/or cytokinin-based PGR treatments influenced three variables: canopy width, branch length increase, and number of branches. Plants treated with topping, topping + coconut water, and topping + BAP showed the best growth performance on canopy width and increased branch length. Treatments of coconut water and BAP responded best to increase the number of branches.

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Evaluation of thermotherapy on potato tubers to control tuber-borne nematodes, *Meloidogyne* spp.

Abstract. Root knot nematodes (*Meloidogyne* spp.) are significant plant-parasitic nematodes frequently transmitted through potato tubers and are a major factor contributing to the decline in both crop quality and yield. This study aims to evaluate the effectiveness of thermotherapy for eliminating *Meloidogyne* spp. from potato tubers and its impact on sprouting viability. Thermotherapy was conducted by immersing potato tubers and second-stage juveniles (J2) of *Meloidogyne* spp. in water at temperatures of 50 °C, 52.5 °C, 55 °C, and 60 °C for durations ranging from 5 to 75 minutes, depending on the treatment. Parameters observed included the mortality rate *of Meloidogyne* spp. J2 and the growth viability of potato seeds. The results showed that thermotherapy applied to second-stage juveniles (J2) of *Meloidogyne* spp. at 50 °C for 20 minutes resulted in complete (100%) nematode mortality. Similarly, the application of thermotherapy to potato tubers at 50 °C for 40 minutes did not significantly affect seed viability. During this treatment, the internal temperature of the tubers, measured at a depth of 1.5 cm, reached the target temperature of 50 °C at the 20-minute mark and was maintained until the 40th minute, ensuring an effective thermal exposure. These results indicate that thermotherapy at 50 °C for 40 minutes represents a safe and effective method for the elimination of *Meloidogyne* spp. in potato tubers. This approach offers a practical and promising strategy to enhance seed health and minimize the risk of nematode dissemination in potato cultivation systems.

Keywords: Heat treatment · *Meloidogyne* spp. · Plant disease control · Potato seeds · Root-knot nematodes

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Introduction

Potatoes (*Solanum tuberosum* L.) are among the most economically valuable and play an important role in global food security (Mishra *et al.*, 2024). However, potato production in many countries, including Indonesia, faces several challenges, one of which is infestation by root-knot *Meloidogyne* spp (Moens et al., 2009; Wesemael et al., 2014; Aprilyani et al., 2015; Utami et al., 2017). These nematodes attack plant roots, inducing gall formation that disrupts the process of water and nutrient absorption by plants, thereby reducing crop yields. The prevalence of *Meloidogyne* spp. is increasing due to its spread through infected potato tubers, particularly in areas with tropical and subtropical climates (Jones et al., 2013).

One of the primary pathways for the spread of *Meloidogyne* spp. is through infested potato tubers. Consequently, managing these nematodes during the post-harvest stage is essential to prevent spread to other production areas (Holajjer et al., 2020; Singh et al., 2020; Nirula et al., 1967; Jatala et al., 1982; Gerič Stare et al., 2022). Various control methods have been developed to suppress populations of these nematodes, including the use of chemical nematicides (Desaeger et al., 2020; Becker et al., 2021; Grabau & Liu, 2021), crop rotation (Win et al., 2016; Mathebula et al., 2024), and resistant cultivars (Chiuta et al., 2021; Iwanaga et al., 2022; Pinheiro et al., 2020; Bali et al., 2021), as well as physical and biological control methods. However, the application of chemical nematicides often raises concerns related to environmental and human health (Pathak et al., 2022), while the effectiveness of crop rotation and resistant cultivars remains limited.

Thermotherapy is a physical control approach that has been applied to various crops for the elimination of pathogens without leaving harmful residues. This method has been used globally for over a century as an environmentally friendly, pre-planting disease management strategy. Among its applications is the control of plant-parasitic nematodes in various planting materials, such as bananas, rice, oranges, vegetable bulbs, and ornamental plants (Lopes et al., 2019). Thermotherapy involves the application of specific temperature via heat, cold, or irradiation to infected plant material, including potato tubers. Heat can be in the form of dry or wet heat. Hot Water Treatment (HWT), which involves soaking planting material in hot water, is a common thermotherapy technique aimed at eradicating nematodes while preserving the viability of the tubers or bulbs (Lopes et al., 2019; Knoetze, 2020; Gu et al., 2022).

Several studies demonstrated that exposing nematodes to elevated temperatures over a specific duration can significantly reduce their populations (Knoetze, 2020; Gu et al., 2022). Hot water immersion techniques have been employed to control various nematode species. For instance, Koen (1969) reported that HWT at 0 °C for 45 to 60 minutes effectively eliminated all developmental stages of *Pratylenchus* spp. in potato tubers, without adversely affecting shoot emergence. In addition to potatoes, this technique has also been applied to other commodities such as garlic (Ahmadi et al., 2019), yam, sweet potato, taro, ginger (Sikora et al., 2018), stroberry (Khanal et al. 2020) and *Syngonium podophyllum* (Lim et al. 2024).

Previous studies have demonstrated that thermotherapy within specific temperature and time ranges can effectively control nematodes in vitro without adversely affecting the quality of bulbs or other plant materials. For instance, temperatures of 45–51 °C for 20–30 minutes have been effective against *Ditylenchus destructor* in garlic (Ahmadi et al., 2019), 50–55 °C for 40 minutes against *Scutellonema bradys* in yam bulbs (Coyne et al., 2010), and a range of 50–55 °C for 10 minutes for *Meloidogyne javanica* as well as 45 °C for 3 hours for *Radopholus similis* on ginger rhizomes (Sikora et al., 2018).

However, information regarding the application of thermotherapy for the control of *Meloidogyne* spp. carried by potato tubers remains limited. The success of thermotherapy is influenced by multiple factors, including treatment temperature and duration, nematode species and developmental stage, the host plant's heat tolerance, and environmental conditions during treatment. Therefore, the application of thermotherapy in potato requires further investigation to establish effective and safe protocols.

This study aims to evaluate the effectiveness of thermotherapy for controlling *Meloidogyne* spp. in potato tubers and to determine the optimal temperature and duration that minimize adverse effects on tuber sprouting viability. It is anticipated that the findings will contribute to the development of sustainable

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and applicable nematode management strategies at the farmer level, particularly in potato production systems.

Materials and Methods

Preparation of potato seed tubers and nematode suspension. Healthy tubers of the Granola G3 variety were used in this study. These tubers were obtained from certified seed producers in Pangalengan, West Bandung Regency, Indonesia. Tubers were selected for uniformity in size (approximately 50 g per tuber; medium size: 31–60 g and storage age (2–3 months), with sprout lengths of approximately 0.5 cm. Root-knot nematode (*Meloidogyne* spp.) suspensions were prepared by extracting nematodes naturally infected potato tubers using the Baermann funnel technique for 48 hours. 15 live and active nematodes (J2) were transferred into a 1.5 mL Eppendorf tube containing 1 mL of distilled water. In addition, healthy potato tubers of uniform size (50–60 g) were prepared for internal temperature measurement tests. These tubers were used to determine the internal temperature at depths of 1.5–2.0 cm from the surface during hot water treatment, using a digital thermometer.

Thermotherapy Procedure for Potato Sprouting Viability and Nematode Mortality. The treatment window, defined as the critical combination of temperature and exposure time, is essential for optimizing thermotherapy in order to preserve potato seed viability and eliminate nematodes. Determining the correct treatment window is crucial because excessive heat exposure can damage tuber physiological quality as seed, whereas insufficient heat exposure may fail to eliminate pathogens effectively. For seed tuber evaluation, healthy potato tubers were first soaked in water at 20–25 °C for 30 minutes to equilibrate initial tuber temperature and reduce thermal shock during treatment, then placed into a holding container. Tubers were subsequently subjected to water bath treatments at temperatures of 50 °C (15–75 minutes), 52.5 °C (15–75 minutes), 55 °C (5–60 minutes), and 60 °C (5–20 minutes). After thermotherapy, tubers were hydrocooled in 20–25 °C water for 10 minutes, air-dried, and transferred into culture boxes maintained at 16–20 °C. Observations on bud whiteness, the number of new buds, and bud size were recorded from day 2 to day 28 post-treatment. Internal tuber temperature at a depth of 1.5 cm was monitored using a digital thermometer.

The treatment window also determines the critical temperature and duration of thermotherapy for nematode mortality. To assess nematode response, an Eppendorf tube containing 1 mL of distilled water and 15 live, active nematodes (10 second-stage juveniles (J2) and 5 adults) was first equilibrated by immersion in water at 20–25 °C. This pre-equilibration step helps standardize the initial conditions before exposure to heat treatments. Subsequently, the tubes were immersed in a water bath pre-set to target temperatures of 50 °C (5–75 minutes), 52.5 °C (5–60 minutes), 55 °C (5–60 minutes), and 60 °C (5–15 minutes). The untreated nematode suspension, maintained at room temperature, served as a negative control. This approach ensures that the observed nematode mortality can be attributed solely to the thermal exposure rather than handling or environmental fluctuations. Observations were made 1 day after hot water treatment by counting the number of dead nematodes. Calculation of nematode mortality with the formula:

$$M=n/N\times100\%$$

Information:
M : Percentage of nematode mortality (%)
n : Number of dead nematodes (tails)
N : Total number of test nematodes (tail)

Results and Discussion

Symptoms of potato tubers infected with *Meloidogyne* spp. The results of the observation of disease symptoms in potato tubers and the identification of the cause are presented in Figure 1. Symptoms observed in potato tubers in the field show early indications of root-knot nematode infection (*Meloidogyne* spp.), characterized by uneven tuber surfaces, root-knot or lumps, non-uniform shape of tubers, and the appearance of premature shoots in some tubers. These physical deformations not only reduce the commercial value of the tubers but also indicate internal physiological disruptions caused by nematode infestation. The symptoms are visually similar to the typical signs of *Meloidogyne* spp. Infestation as reported in various literature (Moens et al., 2009; Wesemael et al., 2014).

To ascertain the type of nematode responsible for these symptoms, morphological identification of the nematodes isolated from tuber tissue and soil around the roots was performed. Microscopic observations revealed juvenile stages (J1, J2, J3, J4) and female morphological characters that matched the description of *Meloidogyne* spp., such as a vermiform-shaped body in J2, a pointed tail, and rounded females with a distinctive perineal pattern. These diagnostic features, consistent with established identification keys (Jones et al., 2013), strengthen the confirmation that the nematodes involved were *Meloidogyne* spp. The alignment between field symptoms and morphological identification results strengthens the suspicion that the nematode that infested potato tubers in this study was *Meloidogyne* spp. This confirmation is important because epidemiologically, the spread of these nematodes can occur using infested tubers as seeds, so it has great potential in spreading to other production areas (Singh et al., 2020).

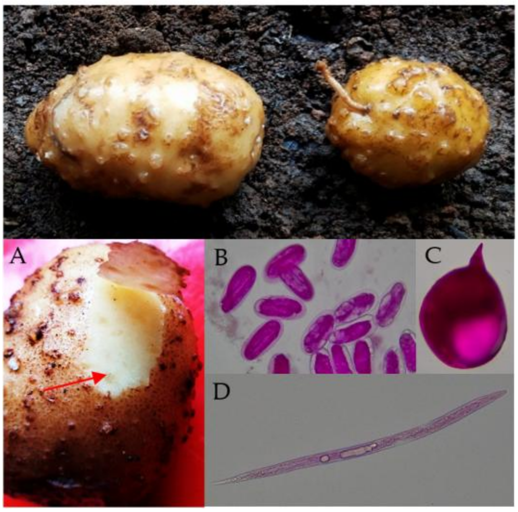

Figure 1. Symptoms of potato tubers infected with *Meloidogyne* spp. and the results of staining of infected parts (a) symptomatic potato tuber incisions show damage to the inside of the tubers, (b) *Meloidogyne* spp. eggs, (c) adult female of *Meloidogyne* spp., (d). juvenile 2 of *Meloidogyne* spp.

Infection by *Meloidogyne* spp. induces physiological disturbances in plants through the formation of giant cells within root tissues, thereby disrupting nutrient and water uptake processes. These pathological changes directly affect tuber development, impacting tuber size, shape, and seed viability (Wesemael et al., 2014; Holajjer et al., 2020). The confirmation of *Meloidogyne* spp. as the causal agent thus provides a critical foundation for the development of effective control strategies, particularly in the postharvest phase, such as thermotherapy treatments aimed at preventing the dissemination of nematodes through seed tubers).

Effects of Thermotherapy on Potato Seeds. Thermotherapy significantly affected potato seed viability, as reflected in shoot number, shoot length, and growth capacity percentage. Overall, increasing the temperature and extending the treatment duration led to a marked decline in seed viability (Table 1).

The viability of potato seeds at 50 °C remained relatively high up to 40 minutes of treatment, with a germination rate of 97.30% and an average bud length of 4.4 cm. However, a significant decline in viability was observed after 45 minutes of exposure, where the germination rate dropped sharply to 53.32%. These results indicate that the physiological heat tolerance threshold of potato seeds at 50 °C lies between 40 and 45 minutes. Measurements of internal tuber temperature at a depth of 1.5 cm showed that temperatures ≥50 °C were reached after 20 minutes and maintained until the end of the 75-minute treatment period, suggesting uniform heat penetration throughout the internal tissues. Following the observations at 50 °C, further evaluation at higher temperatures was conducted to determine the impact of increased thermal exposure on seed viability and to identify the threshold beyond which irreversible physiological damage occurs.

Table 1. Effect of thermotherapy on potato seeds 30 days after treatment (DAT)

Types of Treatment		Number of Buds	Bud Length (cm)	viability (%)	Temperature of potato tubers at a depth of 1.5 cm (°C)	Duration of temperature ≥ 50 ^{o}C (minute)
Temperature (^{o}C)	Duration (minute)					
50	control (0)	4.40 ± 0.55 b	2.96 ± 0.55 a	100.00	25.60	-
	15	4.40 ± 0.55 b	2.82 ± 0.55 b	97.64	48.50	-
	20	4.40 ± 0.55 a	2.80 ± 0.55 b	97.30	49.60	-
	30	4.40 ± 0.55 a	2.90 ± 0.55 a	98.99	50.20	10
	40	4.40 ± 0.55 a	2.80 ± 0.55 a	97.30	50.30	20
	45	3.80 ± 0.45 a	0.60 ± 0.55 a	53.32	50.30	25
	60	3.40 ± 0.55 a	0.36 ± 0.45 a	44.72	50.60	40
	75	2.40 ± 0.55 a	0.40 ± 0.55 a	34.03	50.30	55
52.5	control (0)	4.40 ± 0.55 a	2.96 ± 0.05 a	100.00	25.60	-
	15	4.40 ± 0.55 a	2.68 ± 0.55 a	95.27	50.70	-
	20	4.40 ± 0.55 a	2.42 ± 0.55 a	90.88	50.70	10
	25	4.40 ± 0.55 a	2.42 ± 0.55 a	90.88	50.70	15
	30	4.00 ± 0.71 a	0.80 ± 0.55 a	58.97	50.70	20
	40	1.00 ± 0.00 b	0.32 ± 0.71 a	16.77	51.30	30
	45	1.00 ± 0.71 b	0.16 ± 0.00 b	14.07	51.50	35
	60	0.00 ± 0.00 c	0.00 ± 0.71 b	0.00	52.50	50
	75	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00	52.50	65
55	Control (0)	4.40 ± 0.55 a	2.96 ± 0.05 a	100.00	25.60	-
	5	4.40 ± 0.55 a	2.76 ± 0.55 a	96.62	43.60	-
	10	4.00 ± 0.00 ab	2.44 ± 0.55 a	86.67	49.80	-
	15	3.60 ± 0.55 bc	2.34 ± 0.00 ab	80.44	52.90	5
	20	3.40 ± 0.55 c	1.50 ± 0.55 bc	63.97	53.90	10
	25	1.60 ± 0.55 d	0.54 ± 0.55 c	27.30	54.50	15
	30	0.00 ± 0.00 e	0.00 ± 0.55 d	0.00	54.80	20
	45	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00	55.00	35
	60	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00	55.10	50
60	Control (0)	4.40 ± 0.55 a	2.96 ± 0.05 a	100.00	25.60	-
	5	4.60 ± 0.55 a	3.16 ± 0.55 a	105.65	44.80	-
	10	3.60 ± 0.55 b	1.32 ± 0.55 a	63.21	53.00	-
	15	0.00 ± 0.00 c	0.00 ± 0.55 b	0.00	56.80	5
	20	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00	59.03	10

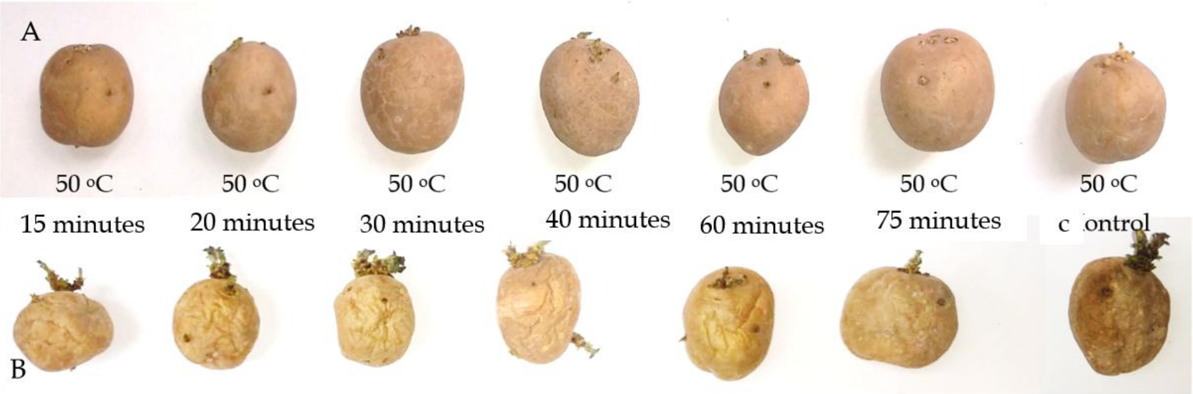

Figure 2. Potato tubers on 50 °C thermotherapy treatment (a) before treatment, (b) 30 days after treatment

Treatment at 52.5 °C resulted in a more rapid decline in seed viability, with a significant reduction observed at 30 minutes (58.97%) and complete loss of viability at 60 minutes (0%). These results indicate that a 2.5 °C increase from 50 °C significantly accelerates damage to the meristematic tissues of the shoots. This decline in viability is consistent with previous studies, which have shown that exposure to elevated temperatures induces protein denaturation and physiological disruptions in seed tissues, ultimately impairing their growth potential (Tang et al., 2018; Singh et al., 2020). Exposure to elevated temperatures can lead to significant physiological damage in plant tissues, including the disruption of cellular membrane integrity, denaturation of essential enzymes, and the collapse of mitochondrial function, which impairs energy production required for cell division and growth (Park et al, 2024; Wahid et al., 2007). Additionally, high temperatures can cause oxidative stress through excessive reactive oxygen species (ROS) production, leading to lipid peroxidation and DNA damage, further compromising seed viability and regenerative capacity.

Exposure to temperatures of 55 °C and 60 °C resulted in a substantial and accelerated reduction in potato seed viability. At 55 °C, viability declined to 0% after 30 minutes, while at 60 °C, complete loss was recorded within only 15 minutes of treatment. Early physiological damage was evident through the absence and shortening of emerging buds prior to total mortality. The rapid attainment of internal tuber temperatures ≥50 °C within 10–15 minutes at these higher external temperatures likely contributed to accelerated tissue degradation. Consistent with previous findings, excessive thermal exposure compromises meristematic and vascular tissues, disrupts mitochondrial integrity, and induces protein denaturation, thereby impairing cellular respiration and division processes (Tang et al., 2018; Wahid et al., 2007; Singh et al., 2020). Although a brief exposure at 60 °C for 5 minutes slightly enhanced sprouting relative to the control, the effect was insufficient for achieving complete nematode elimination. Collectively, these findings suggest that thermotherapy at 50 °C for 40 minutes represents a critical threshold, ensuring effective internal heating (≥50 °C for ≥20 minutes) necessary for nematode control while maintaining seed viability above 95% (Holajjer et al., 2020; Shurtleff & Averre, 2000).

Effect of Thermotherapy on Juvenile Mortality of *Meloidogyne* spp. The results of the thermotherapy treatments showed a significant effect on the mortality of second-stage juveniles (J2) of *Meloidogyne* spp. at 24 hours post-treatment, as presented in Table 2. Increased temperature and length of treatment duration consistently increase nematode mortality rates. J2 mortality reaches 100% in almost all temperature and duration combinations above the 50 °C temperature threshold.

Table 2. Effect of thermotherapy on mortality of J2 *Meloidogyne* spp. at 24 hours after treatment (HAT)

Types of Treatment		Mortality (%)			
Temperature (°C)	Duration (minute)				
	control	0.00	±	0.00	a
50	5	60.00	±	0.71	b
	10	78.67	±	0.84	c
	15	98.67	±	0.45	d
	20	100.00	±	0.00	d
	30	100.00	±	0.00	d
	40	100.00	±	0.00	d
	45	100.00	±	0.00	d
	60	100.00	±	0.00	d
	75	100.00	±	0.00	d
	control	0.00	±	0.00	a
52.5	5	76.00	±	1.14	b
	10	86.67	±	1.00	c
	15	100.00	±	0.00	d
	20	100.00	±	0.00	d
	25	100.00	±	0.00	d
	30	100.00	±	0.00	d
	40	100.00	±	0.00	d
	45	100.00	±	0.00	d
	60	100.00	±	0.00	d
	75	100.00	±	0.00	d
	control	0.00	±	0.00	a
55	5	89.33	±	0.89	b
	10	100.00	±	0.00	c
	15	100.00	±	0.00	c
	20	100.00	±	0.00	c
	25	100.00	±	0.00	c
	30	100.00	±	0.00	c
	45	100.00	±	0.00	c
	60	100.00	±	0.00	c
	control	0.00	±	0.00	a
60	5	100.00	±	0.00	b
	10	100.00	±	0.00	b
	15	100.00	±	0.00	b

At 50 °C, the J2 mortality rate increases with the length of the treatment time. Initial mortality of 60% occurred after 5 minutes and increased to 78.67% at 10 minutes. Mortality reached 98.67% at 15 minutes and 100% after 20 minutes of treatment. This suggests that J2 *Meloidogyne* spp. has a low heat tolerance threshold, and a temperature of 50 °C for at least 20 minutes is enough to kill all J2 individuals in the sample. Treatment at 52.5 °C showed higher effectiveness, with a mortality of 76% in just 5 minutes, and reached 100% at a duration of 15 minutes. Similarly, at 55 °C, mortality reached

89.33% within 5 minutes and 100% after 10 minutes. Meanwhile, a temperature of 60 °C killed 100% of J2 in just 5 minutes, suggesting that high temperatures significantly accelerate physiological damage to nematodes, possibly through protein denaturation and disruption of cellular metabolism (Perry & Moens, 2024).

The effectiveness of thermotherapy in eliminating nematodes supports previous findings indicating that temperatures between 50–55 °C for a specific duration are effective in inactivating the eggs and juvenile stages of the nematode *Meloidogyne* spp. (Sikora & Fernandez, 2018; Holajjer et al., 2020). In the context of nematode management in potato tubers, thermotherapy treatment can be applied as an environmentally friendly, non-chemical alternative method to prevent the spread of nematodes through infected tuber seeds. However, it is important to note that effective high-temperature treatment in killing nematodes can also potentially impair the viability of potato tubers, as shown in previous data. Therefore, selecting an appropriate combination of temperature and duration must balance the efficacy of nematode elimination with the suitability of the tubers for use as seed. In this study, treatment at 50 °C for 20–40 minutes maintained tuber sprouting capacity above 95% and resulted in internal temperatures reaching 50 °C at a depth of 1.5 cm. This temperature–time range effectively caused 100% mortality of second-stage juveniles (J2) at 50 °C for 20 minutes (Table 2).

Conclusion

The application of thermotherapy at 50 °C for 20 minutes effectively eliminated second-stage juveniles (J2) of *Meloidogyne* spp., achieving complete (100%) mortality. Moreover, treating potato tubers at 50 °C for 40 minutes did not significantly affect sprouting viability, as the internal temperature at a depth of 1.5 cm reached and sustained the target temperature during the treatment period. These findings demonstrate that thermotherapy at 50 °C for 40 minutes is a safe and effective method for the elimination of *Meloidogyne* spp. in potato tubers. Therefore, this approach can serve as a practical and reliable strategy to improve seed health and reduce the risk of nematode transmission in potato production.

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The effect of soil conditioner and growth booster on the agronomic characters and number of nodules of black soybean

Abstract. Soil Conditioner & Growth Booster (SCGB) is a potion that was made to be used as a soil conditioner and plant growth promoter. SCGB is made from organic matter, biological fertilizers, soil ameliorants, and microelements. This research aims to determine the best dosage of SCGB on the agronomic characters, effective nodules, and black soybean variety 'Detam-1' yield. This research was conducted in the experimental field of the Faculty of Agriculture, Winaya Mukti University, Sumedang, Indonesia. This experiment used a simple randomized block design (RBD) with six treatments (0, 3, 6, 9, 12, 15 kg/ha) and repeated 4 times. The observation was conducted on a number of effective root nodules, plant height, number of leaves, 100-grain weight, number of pods, seed weight per plant, and seed weight per plot. The result showed that SCGB application significantly improved agronomic characteristics of black soybean plants, increasing the number of effective nodules by 147% to 168% compared to untreated plants. A dose of 3 kg/ha SCGB showed the best results in increasing 35% on yield seed weight per hectare compared to without application of SCGB.

Keywords: Agronomic performance · Black soybean · Effective nodule · SCGB

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Introduction

Black soybean is one of the soybean varieties (*Glycine max* (L.) Merr.) used as raw materials for healthy drinks and foods. This variety is used for obesity and hyperglycemia diets because it has a high protein and low-fat content and contains antioxidants, namely anthocyanins and isoflavones (Li et al., 2024; Ganesan & Xu, 2017; Mitharwal et al., 2024; Yamashita et al., 2024). Black soybeans are generally used by the soy sauce industry in Asia countries (Ginting, 2021). Amino acids and volatile compounds in black soybeans affect the unique taste of soy sauce (Shin et al., 2019).

Many farmers cultivate soybeans using synthetic nitrogen, phosphorus, and potassium fertilizers without balancing with organic fertilizers and micronutrients. Cultivation like this not only endangers plant health but can also reduce the soil's physical, chemical, and biological quality. Therefore, we must maintain plant and soil health by adding micro fertilizers, soil ameliorants, organic fertilizers, and biofertilizers.

In small concentrations, micro fertilizers, such as boron (B), manganese (Mn), and zinc (Zn), have various benefits for plants. Boron affects soybean germination, growth, and yield (Dameto et al., 2023). Manganese can increase soybean resistance to stress (Jiang et al., 2023). Zinc not only increases soybean resistance to stress but also fortifies soybean seeds so that people do not experience symptoms of Zn deficiency (Dai et al., 2020).

Soil ameliorants can maintain soil quality, which can ultimately maintain crop yields. It can be in the form of biochar, which helps increase soil porosity, increase soil aggregate stability, increase water availability, increase cation exchange capacity (CEC), and increase root nodules and soybean plant yields (Blanco-Canqui, 2017; Domingues et al., 2020; Turmuktini et al., 2020; Turmuktini et al., 2022). Humic acid and dolomite have almost the same function and increase soybean yields (Ampong et al., 2022; Lenssen et al., 2019; Pimolrat et al., 2020; Takamoto et al., 2023). Organic fertilizers can increase soil nutrients, as nutrients for beneficial soil microorganisms, and improve soil physical properties, increasing soybean growth and yields (Assefa & Tadesse, 2019; Onyenali et al., 2020). Several organic materials can be used as fertilizers, such as sugarcane filter cake, guano, and palm ash (Marwa et al., 2021; Dotaniya et al., 2016; Zahrah & Kustiawan, 2022).

Biofertilizers are known to increase soybean growth and yield. Phosphate-solubilizing bacteria can dissolve unavailable P, increasing soybean productivity (Shome et al., 2022). Nitrogen-fixing bacteria can provide N for soybeans, thereby reducing the dose of N fertilizer and increasing crop yields (Cordeiro & Echer, 2019).

The enormous benefits of micro fertilizers, soil ameliorants, organic fertilizers, and biofertilizers for soybean plants prompted the research team to create a combination of these materials called Soil Conditioner and Growth Booster (SCGB). These materials consist of micro fertilizers (B, Mn, and Zn), soil ameliorants (coconut shell biochar, dolomite, and humic acid), organic fertilizers (sugarcane filter cake compost, guano fertilizer, and palm ash), and biological fertilizers (phosphate-solubilizing bacteria and nitrogen-fixing bacteria). SCGB is expected to increase the effective nodules, growth, and yield components, and yield of black soybeans.

Materials and Methods

The research was conducted from March to June 2023 at the experimental field of the Faculty of Agriculture, Winaya Mukti University, Sumedang Regency, West Java Province, Indonesia, with an altitude of 850 meters above sea level (asl) and rainfall type C (slightly wet). The soil has criteria for moderate N and P content, while the K content is low but has a high CEC (Table 1). The laboratory equipment used is an analytical balance, ruler, oven, grain moisture meter, and plant cultivation tools in the field. The materials used are soybean seeds of 'Detam-1' variety; SCGB (the mixture consists of B, Mn, Zn, coconut shell biochar, dolomite, humic acid, sugarcane filter cake compost, guano fertilizer, palm ash, phosphate-dissolving bacteria, and nitrogen-fixing bacteria) (Table 2); rice straw compost as a SCGB diluent; urea; SP-36; and KCl. Botanical pesticides in the form of garlic extract, pesticides with active ingredients Profenofos 500 EC, lambda-cyhalothrin 106 g/l + thiamethoxam 141 g/l, Fipronil 50 SC, Propineb 70 WP, carbofuran 3G, and brofflanilide 53 SC were used alternately to control pests and diseases that attack plants.

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Table 1. Results of soil analysis at the experimental field

Parameter	Value	Unit	Criterion
pH (HCl 25%)	6.1	mg/100 g	Slightly acid
Organic-C	2.16	%	Medium
Total N	0.30	%	Medium
C/N	7.20		Low
P_2O_5 (HCl 25%)	35.00	mg/100 g	Medium
K_2O	18.00	mg/100 g	Low
CEC	37.24	cmol/kg	High
Ca-exchange	7.16	cmol/kg	Medium
Mg-exchange	2.12	cmol/kg	High
K-exchange	0.35	cmol/kg	Low
Na-exchange	0.0054	cmol/kg	Very low

Note: Soil fertility criteria based on Balittanah (2009)

The experiment used a randomized block design (RBD) composed of 6 SCGB dose treatments, namely 0 (A), 3 (B), 6 (C), 9 (D), 12 (E), and 15 kg/ha (E), which were repeated four times. Data were analyzed using variance analysis (ANOVA), and testing was continued using Duncan's Multiple Range Test at a 5% significance level. Correlation analysis was conducted on the number of effective root nodules, yield components, and black soybean yields using the Pearson correlation test at a 5% significance level.

Table 2. The profile of soil conditioner & growth booster (SCGB) characteristics

Parameter	Value	Unit
Organic-C	19.56	%
C/N	19	
Moisture Content	14.96	%
N	1.02	%
P_2O_5 (HCl 25%)	6.28	%
K_2O	0.52	%
Ca	7.16	%
Mg	2.19	%
CEC	35.02	cmol kg^{-1}

Note: Measurement based on Balittanah (2009) protocol

The experiment began with land preparation, including soil cultivation and making plots measuring 1 m x 1 m, with a distance between treatments and replications of 30 cm each. SCGB was first mixed with 2 tons of rice straw compost ha-1 according to the treatment dose, then inserted into the planting hole with a distance of 25 x 25 cm. The seeds were coated with 20 g/kg biofertilizer until it adhered evenly to the seeds, and then two seeds were planted per hole. One week later, the seeds were thinned to 1 seed per hole. The fertilizers given were 50 kg/ha urea, and 75 t/ha KCl and SP36 when the plants were 15 days after sowing (DAS) (half dose urea, SP36, and KCl), and 35 DAS (continued half dose urea). Another maintenance is weeding, and pest and disease control is carried out when weeds grow, or there are pest and disease attacks. Harvesting occurred after physiological maturity characteristics appeared, namely dry brown pods and hard seeds with a maximum water content of 25%. Observations were made on the number of effective nodules, the percentage increase in effective root nodules, agronomic characters, and plant yields. Effective root nodules are root nodules that can fix nitrogen, as indicated by the pink color of the nodules (Jin et al., 2022). The percentage increase in effective root nodules is the difference between the effective root nodules given SCGB and the control in percentage form. The agronomic characteristics observed were plant height and number of leaves (as growth components); number of pods per plant and weight of 100 grains (as yield components). Plant height and number of leaves were observed at 3, 4, 5, and 6 weeks after planting (WAS), while the number of pods and weight of 100 grains were observed at harvest. Plant yields were observed at harvest: seed weight per plant and seed weight per plot.

Results and Discussion

Results. The statistical analysis showed that the SCGB dose significantly affected the number of effective nodules and effective nodules increase (%). The 3-15 kg/ha SCGB treatment gave more effective nodules than the control (Table 3).

SCGB also showed a significant difference compared to the control on the growth of black soybeans, namely in height and number of leaves (Table 4). The 3 kg/ha dose gave a higher plant height than the control at ages 3, 4, and 5 WAS. A higher dose (6-15 kg/ha) could only give a difference in height at age 6 WAS. In contrast, almost all SCGB doses showed a difference in the number of leaves at ages 3-6 WAS, but the 3 kg/ha dose showed a difference in the number of leaves at age 6 WAS.

The components of the black soybean plant yield were also influenced by SCGB (Table 5).

The number of pods per plant increased after being given 3-15 kg/ha SCGB, but the highest number of pods was given by a dose of 3 kg/ha, although it was not different from 6 and 9 kg/ha. The heaviest 100-grain weight was also given by a dose of 3 kg/ha SCGB but was not different from 6, 9, and 15 kg/ha. The increase in yield components due to SCGB also increased crop yields. The heaviest seed weight per plant was given by 3-9 kg SCGB. Doses of 3 and 9 kg/ha gave the highest seed weight per plant but were not significantly different from 6 kg/ha. In the correlation analysis, the number of root nodules affected the growth components (plant height and number of leaves), yield components (number of pods and 100-grain weight), and also black soybean yield (seed weight per plant). Plant height and number of leaves as growth components affected all yield components and crop yields. Seed weight per plant as a crop yield also affected other crop yields, namely seed weight per plot (Table 6).

Table 3. The effect of soil conditioner & growth booster (SCGB) application on the number of effective nodules and effective root nodules increase (%)

Treatments	Number of Effective Nodules	Effective Nodules Increase (%)
A (0 kg/ha)	2.25 a	0 a
B (3 kg/ha)	5.56 b	147 b
C (6 kg/ha)	5.50 b	145 b
D (9 kg/ha)	5.75 b	155 b
E (12 kg/ha)	6.05 b	168 b
F (15 kg/ha)	6.00 b	166 b

Note: The average values marked with the same lowercase letter in the same column indicate no significant difference according to Duncan's Multiple Range Test at a 5% significance level.

Table 4. Effect of soil conditioner & growth booster (SCGB) application on black soybean plant height and number of leaves from 3-6 weeks after sowing (WAS)

Treatments	Plant Height (cm)			
	3 WAS	4 WAS	5 WAS	6 WAS
A (0 kg/ha)	14.20 a	18.10 a	21.76 a	28.63 a
B (3 kg/ha)	16. 10 b	20.50 b	25.20 c	34.55 b
C (6 kg/ha)	15.21 ab	19.25 ab	23.50 abc	33.75 b
D (9 kg/ha)	15.05 ab	19.48 ab	23.51 abc	32.79 b
E (12 kg/ha)	15.17 ab	19.60 ab	22.70 ab	32.58 b
F (15 kg/ha)	15.19 ab	19.85 ab	24.21 bc	32.72 b
	Number of Leaves			
	3 WAS	4 WAS	5 WAS	6 WAS
A (0 kg/ha)	2.38 a	3.50 a	4.46 a	9.42 a
B (3 kg/ha)	2.70 b	4.10 c	6.66 b	13.56 b
C (6 kg/ha)	2.79 b	3.85 b	6.00 b	12.04 ab
D (9 kg/ha)	2.55 b	3.91 b	6.19 b	11.80 ab
E (12 kg/ha)	2.66 b	3.68 ab	5.89 b	11.06 ab
F (15 kg/ha)	2.70 b	3.84 b	5.94 b	11.08 ab

Note: The average values marked with the same lowercase letter in the same column indicate no significant difference according to Duncan's Multiple Range Test at a 5% significance level.

Table 5. Effect of soil conditioner & growth booster (SCGB) application on soybean plant yield components

Treatments	Number of Pods	100-grain weight (g)	Seed Weight per Plant (g)	Seed Weight per Plot (g)
A (0 kg/ha)	25.63 a	11.60 a	7.84 a	268.67 a
B (3 kg/ha)	50.18 d	14.90 c	13.70 c	362.57 c
C (6 kg/ha)	49.59 d	13.50 bc	12.98 c	331.51 abc
D (9 kg/ha)	41.20 cd	13.34 bc	13.66 c	350.50 c
E (12 kg/ha)	37.19 bc	12.05 ab	9.68 ab	281.39 ab
F (15 kg/ha)	35.63 b	13.10 bc	10.23 b	281.94 ab

Note: The average values marked with the same lowercase letter in the same column indicate no significant difference according to Duncan's Multiple Range Test at a 5% significance level.

Table 6. Correlation matrix between the number of root nodules, growth components, and yield components of black soybean

	Number of Effective Nodules	Plant Height	Number of Leaves	Number of Pods	Seed Weight per Plant	Seed Weight per Plot
Number of Effective Nodules	1					
Plant Height	0.619*	1				
Number of Leaves	0.517*	0.851*	1			
Number of Pods	0.659*	0.535*	0.434*	1		
Seed Weight per Plant	0.600*	0.509*	0.442*	0.841*	1	
Seed Weight per Plot	0.399	0.512*	0.486*	0.703*	0.796*	1

Note: correlation values followed by an * indicate a significant correlation at the 5% significance level.

Discussion. SCGB treatment can increase the number of effective root nodules of black soybeans. The increase in nodules is because SCGB contains nitrogen-fixing bacteria. With a supportive environment, inoculation of nitrogen-fixing bacteria that ar symbiotic with soybeans can increase the number of effective root nodules (Gebremariam & Tesfay, 2021; Argaw, 2016; dos Santos Sousa et al., 2022). This increase could provide sufficient nitrogen supply for plants (Singh et al., 2022).

SCGB also increases plant height and the number of leaves in black soybeans. Sufficient nitrogen from effective root nodules can be used as a component of proteins and enzymes in the plant body to improve its growth (Oliveira et al., 2017; Gou et al., 2023). Nitrogen also forms chlorophyll in leaves, increasing the number of leaves (Ye et al., 2025). The significant correlation between the number of effective root nodules with plant height, and the number of leaves also evidences growth increase. In addition to nitrogen-fixing bacteria, phosphate-solubilizing bacteria can improve growth by providing energy (Pan & Cai, 2023). However, this study did not observe phosphate uptake, so further research is needed.

Organic fertilizers also provide various nutrients for growth (Table 2). Organic matter can be energy for microorganisms that are good for plants, including phosphate-solubilizing bacteria (Gunina & Kuzyakov, 2022). Organic fertilizers and soil ameliorants increase the cation exchange capacity, providing various nutrients for plants (Cooper et al., 2020). This material can also improve the physical properties of the soil. However, this study has not revealed whether the soil's physical properties have improved after SCGB application.

Micro fertilizers, such as B, Mn, and Zn contained in SCGB, can increase plant growth with a small dose, while too high a dose can cause poisoning (Kaur & Garg, 2021; Santos et al., 2017; Landi et al., 2019). The possible toxicity of micro fertilizer caused SCGB doses of more than 3 kg/ha to increase plant height no longer, even though the number of leaves was the same as the control. The increase in the number of effective nodules and growth components also causes the yield and yield components to increase. This is evidenced by the significant correlation between the number of effective nodules and growth components with the yield and yield components, except for the number of effective nodules with seed weight per plot. The number of effective nodules likely affects seed weight per plot more indirectly than directly, namely through the effect on growth components, yield components, and seed weight per plant.

Conclusion

SCGB dose of 3 kg/ha gave the best number of effective nodules, yield components, and crop yield compared to the control. This research must be continued to determine the effect of SCGB on nutrient absorption, soil physical properties, and toxicity of microelements B, Mn, and Zn.

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