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PREFACE

Kultivasi Journal Volume 23 No. 3 (2024) contains articles from various scientific fields in the agricultural field. These articles focus on plant production and are conducted from various sectors, such as plant breeding, agronomy, plant pests and diseases, weed science, and soil science. The knowledge of agricultural techniques in this edition also elevated the industry in the AI era as the improving in all sectors.

This publication still consists of 14 articles from academicians and researchers edited and reviewed by professional editors and reviewers from local and overseas. These are expected to increase academic knowledge for academic scientists and agricultural practitioners. The contribution of researchers and academics is an essential factor in the future development of the farming industry. Kultivasi Journal only accepts and publishes an English version to spread our information more widely domestically and internationally to readers and authors. We are still preparing to get International journal indexing. We hope that sharing knowledge through the Kultivasi journal can always be useful and become the main of future agricultural progress.

Warm greetings from the Kultivasi Journal Editor.

AUTHOR'S INSTRUCTIONS

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

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

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

Special Requirements

Review Articles:

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

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

Research Articles:

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

Title

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

Author's name

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

Abstract

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

Introduction

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

Materials and Method

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

Results and Discussions

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

Conclusion

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

Acknowledgment

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

Reference

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

- Two authors: Tati Nurmala and Yudithia Maxiselly *then written* Nurmala and Maxiselly (2014) or (Nurmala and Maxiselly, 2014).
- Three or more authors: Nurmala et al. (2014) or (Nurmala et al., 2014).
- Book writing format: Author's Last Name. Initials of the year it was published. Book title (each initial letter of a word is written in capital letters, except for conjunctions/prepositions; Edition if the edition is more than one). Name Publisher. Place published.
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  Example: Zakry FAA, Shamsuddin ZH, Rahim KA, Zakaria ZZ, Rahim AA. 2012. Inoculation of Bacillus sphaericus UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the 15N isotope dilution technique. Microbes Environ., 27: 257–262.

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Priyanto SB · Herawati · Suwarti · Ar Rahman A · Andayani NN · Fattah A · Azrai M

Maize hybrids' genetic variability based on qualitative and quantitative traits

Abstract. Genetic variability was a prerequisite to doing a plant breeding program. A broad genetic variability allows plant breeders to select a desired genotype. This research aims to assess the maize hybrid's genetic variability based on qualitative and quantitative traits. This research was conducted in the Bone district, south Sulawesi, from November 2022 to March 2023. Fifteen maize hybrids were arranged in a randomized complete block design with three replications. The variables observed are qualitative and quantitative traits. Principal component (PCA) and cluster analyses assessed the genetic variability. The result indicated that based on a loading factor greater than 0.70, the qualitative traits such as intensity of green color, anthocyanin coloration of brace roots, length of lateral branch, intensity anthocyanin coloration of silk, and degree of zigzag displayed high variability. quantitative like days to anthesis, days to silk, leaf length, 1000 seeds weight, yield, ear diameter, number of row seeds per ear, ear height, ear length, and number of seeds per row also exhibit high variability. Cluster analysis shows a broad genetic variability on qualitative and quantitative traits demonstrated by Euclidean levels 6.68-10.93 and 3.43-5.08, respectively, and generated the dendrogram that divides genotypes into four main clusters for qualitative and five for quantitative traits.

Keywords: Genetic variability · Maize hybrid · Qualitative traits · Quantitative traits

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Introduction

Maize is one of the world's most essential crops. Maize kernels contain water (10.49±0.01%), ash (1.45±0.01%), protein (11.78±0.05%), fat (5.59±0.22%), crude fiber (6.84±0.07%), total carbohydrates (70.69±0.21%) and energy of (380.19±1.56 kcal/100g) based on dry weight (Murningsih et al., 2019; Rouf Shah et al., 2016). In addition to its use as human foodstuffs, livestock feed, chemical goods, and biofuels, maize is a source of life and prosperity for people in several countries. It is expected that, in terms of production and trade, maize—currently the most-produced cereal—will surpass all other crops in the next ten years. The developing world's need for maize will double (Erenstein et al., 2022). Hence, maize yield must be enhanced to satisfy these demands.

The enhancement of maize yield can be achieved by developing a novel high-yield maize cultivar. Novel high-yield maize cultivar development relies on genetic variability (Kotschi & Horneburg, 2018; Mengistu et al., 2020) Genetic variability is population variation among members (Litrico & Violle, 2015) When genetic variability is broad, plant breeders can combine the desired traits to develop novel varieties (Ahmar et al., 2020; Swarup et al., 2021). A comprehensive understanding of the germplasm's genetic variability is needed to recombinate traits correctly.

Maize is a plant with board variability. Genetic variability can be estimated using qualitative and quantitative traits (Alemu et al., 2020). Qualitative traits in maize are typically controlled by one or a few genes and exhibit discrete variations. These traits are often used to identify and classify maize varieties and hybrids. Qualitative traits provide easy markers for initial selection, while quantitative traits are complex and influenced by multiple genes, requiring advanced breeding techniques to achieve desired improvements. The range of trait variability in plant genetic materials is an excellent resource for plant breeders to develop and improve new varieties with desired traits (Darrudi et al., 2018). Because of this, breeding programs need to look at how different quantitative and qualitative traits are in genetic resources (Bhadmus et al., 2022; Bhandari et al., 2017; Wang et al., 2023).This information helps plant breeders develop optimal breeding strategies for breeding populations.

Principal component analysis (PCA) and clustering analysis are commonly used to measure genetic variability. PCA reduces data dimensionality by transforming it into principal components, effectively estimating population variability based on traits. Studies on upland rice (Tuhina-Khatun et al., 2015), wild cassava (Karuniawan et al., 2017), sunflower (Dudhe et al., 2020), and alfalfa (Sayed et al., 2022) have utilized PCA to determine genetic variability. In maize, PCA was used to identify key traits for breeding drought-resistant varieties (Esen et al., 2022) and identified the heritability and genetic variability traits like grain yield, kernels per ear, ear diameter, and thousand kernel weight (Matin et al., 2022; Rai et al., 2021; Yadesa et al., 2022). PCA with SNP data was used by Ayesiga et al., (2023) to identify genetically of distinct maize inbred lines. Clustering analysis groups genotypes based on similarities, visualizing these relationships in dendrograms for easier understanding. This research aims to assess genetic variability in maize hybrids using qualitative and quantitative traits, providing insights for developing maize breeding programs.

Materials and Methods

Research Site. This research was conducted between November 2022 and March 2023 in the Bone district of South Sulawesi. The location is situated at a latitude of 5.06607°S and a longitude of 120.2120°E. This site is dryland with Latosol soil at an altitude of 80 m above sea level and has a D2 climate type according to Oldeman & Frere, (1982)

Plant Materials. Fifteen hybrids were used in this research. Thirteen conventional crosses maize, including TH 1- TH 13, and two commercial hybrids that have high yields and are widely adopted, NK 6172 and P 32, are used as check varieties (Table 1).

Research Methods. This study used a Randomized Complete Block Design (RCBD) with three replications. Each plot had four rows, each 5 meters long. The spacing between rows was 0.70 m, and the spacing between individual maize plants within a row was 0.20 m. Initially, two seeds were sown per hill, but after two weeks, seedlings were thinned into one per hill to maintain a plant density of 71, 428 plants per hectare, allowing only one plant per stand to grow. Standard agricultural practices were applied for field maintenance, following recommended guidelines.

Table 1. The maize material used in this experiment.

No	Code	Origin	
1	TH 1	LG 1 X G 222	Tested variety
2	TH 2	MP 2 X LG 1	Tested variety
3	TH 3	LN 64 X LG 1	Tested variety
4	TH 4	LN 86 X G 222	Tested variety
5	TH 5	B 992 X MP 2	Tested variety
6	TH 6	SB 35 X MP 4	Tested variety
7	TH 7	RE 71 X G 222	Tested variety
8	TH 8	IM 33 X RS 261	Tested variety
9	TH 9	HF 22 X G 222	Tested variety
10	TH 10	G 222 X MP 2	Tested variety
11	TH 11	MT 20 X CL 14	Tested variety
12	TH 12	LN 18 X HF 22	Tested variety
13	TH 13	RS 122 X G 222	Tested variety
14	NK 6172	NK 6172	Check Variety (commercial hybrid)
15	P 32	P 32	Check Variety (commercial hybrid)

Data Collections. The observed variables were divided into two types: qualitative and quantitative traits. The qualitative traits consisted of various traits, such intensity of green color (IGC), undulation of margin of blade (UMB), attitude of blade (AB), degree of zigzag (DZ), anthocyanin coloration of brace roots (ACBR), anthocyanin coloration of internodes (ACI), anthocyanin coloration of silks (ACSi), intensity anthocyanin coloration of silk (IACS), anthocyanin coloration of sheath (ACSh), anthocyanin coloration at base of glume (ACBG), anthocyanin coloration of glume excluding base (ACGEB), anthocyanin coloration of fresh anthers (ACFA), density of spikelet (DS), shape of tassel (ST), angle between main axis and lateral branches (ABMALB), number of primary lateral branches (NPLB), attitude of lateral branches (ALB), length of main axis above lowest lateral branch (LMAALLB), length of main axis above highest lateral branch (LMAAHLB), length of lateral branch (LLB), and shape of stem (SS). The quantitative traits included days to flowering, growth traits, yield components, and yield. Days to flowering consist of days to anthesis (DA) and days to silk (DS). Meanwhile, growth traits comprise plant height (PH), ear height (EH), ear diameter (SD), leaf length (LL), leaf width (LW), leaf angle (LA),. The yield component contains moisture content (MC), ear length (EL), ear diameter (ED), number of row seeds per ear (NRE), number of seeds per row (NSR), 1000 seeds weight (W 1000), shelling percentage (SP), fresh ear weight (FEW), yield (Y).

The qualitative trait and days to flowering were observed 55 days after planting (DAP). The growth traits were observed at 85 DAP in ten plant samples. Harvest was done at the two middle rows of plants at 105 DAP, where a black layer at the base of kernel seeds appeared, indicating that the seeds were physiologically mature. Ears were collected from two middle rows of each plot. The yield component was observed at ten ear samples. The yield (Y) was corrected to 15% moisture and converted to t/ha. A digital scale was utilized to weigh each genotype yield.

Data Analysis. The genetic variability was investigated using a multivariate analysis method with Principal Component Analysis, and the level of similarity between varieties was measured with cluster analysis. The data was processed using Euclidean distance and the standardized Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and shown as a dendrogram. A variable with a loading factor greater than 0.7 is identified as a variable that contributes to diversity (Jollife & Cadima, 2016). This analysis used Numerical Taxonomy and Multivariate System (NTSYS) software version 2.02 (Rohlf, 2000) and IBM SPSS Statistic version 23 (IBM, 2015).

Results and Discussion

The genetic variability of qualitative and quantitative traits in this study was determined using the PCA method. PCA is a multivariate technique that reduces the dimensionality of original variables that are highly correlated into mutually independent variables called Principal Components (PC). This method's maximum

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number of principal components is equal to the number of original variables(Agustina & Waluyo, 2017). Each PC represents the percentage of variance a given variable contributes to the overall variance (Aristya et al., 2017). The eigenvalue indicates the magnitude of the contribution of PC to the total variance. The first PC (PC1) has the highest eigenvalue and the most significant proportion of variance, while the subsequent PCs have lower eigenvalues than PC1 and explain the remaining variance (Pachauri et al., 2017).

Many approaches to determining the number of PCs are necessary to explain the minimum total variability. This study determines the criteria for PCs with eigenvalues higher than one (Woolford, 2015). A loading factor expresses the correlation between the measured variables and the PC. The higher the loading factor's value, the closer the variable correlation is to the PC (Hefny et al., 2017). Variables that significantly contribute to variability are identified by a loading factor value exceeding 0.70 (Kaiser, 1974).

Genetic variability based on the qualitative trait. The PCA identified six principal components with eigenvalues greater than one based on qualitative traits. These components collectively explain 83.07% of the total variability (Table 2). The identified PCA could explain almost all the variability in qualitative traits Only 16.93% of the variability in qualitative traits is not explained by these principal components. Huqe et al (2021) reported that the total diversity represented by a PC with an eigenvalue of more than one is sufficient to describe diversity in a population.

PC 1 provides 23.70% of the total variability and has an eigenvalue of 4.98. This PC is composed of the intensity of green colour, anthocyanin coloration of brace roots, and length of lateral branch with loading factor values 0.74, 0.80 and 0.74 respectively. The second PC (PC2) had an eigenvalue of 3.68, explaining 17.53% of the variability. It was strongly associated with one trait: the intensity of anthocyanin coloration of silk (loading factor= -0.72). The eigenvalue and contribution to the total variation of PC3 are 3.11 and 14.79. The length of main axis above highest lateral branch with a loading factor -0.82 was a variable associated with PC 3. The degree of zigzag(loading fac tor=-0, 79) affects the variation at PC 4, which is 12.93%. The fifth PC (PC5) and

Table 2. Eigenvalues, variation explained (%), cumulative variance (%), and loading factor of qualitative trait.

Trait and Component	Principal Component					
	1	2	3	4	5	6
Intensity of green colour	**0.74**	0.38	0.29	0.32	-0.25	-0.01
Undulation of margin of blade	0.32	0.30	-0.52	-0.32	0.24	0.49
Attitude of blade	-0.32	-0.54	-0.19	0.51	0.17	0.37
Degree of zigzag	0.01	0.24	-0.17	**-0.79**	-0.05	0.16
Anthocyanin coloration of brace roots.	**0.80**	0.12	0.14	0.33	-0.06	0.10
Anthocyanin coloration of internodes	0.54	0.57	0.46	0.25	-0.01	0.15
Anthocyanin coloration of silks	-0.52	0.66	-0.17	-0.16	-0.31	-0.20
Intensity anthocyanin coloration of silk	0.15	**-0.72**	0.21	0.34	0.33	0.23
Anthocyanin coloration of sheath	0.35	0.39	0.69	-0.01	-0.23	0.02
Anthocyanin coloration at base of glume	0.11	0.43	-0.17	-0.07	0.69	-0.27
Anthocyanin coloration of glume excluding base	-0.15	-0.41	0.62	0.05	0.26	-0.45
Anthocyanin coloration of fresh anthers	0.64	0.30	0.10	-0.41	0.42	-0.15
Density of spikelet	-0.64	0.39	0.27	-0.11	0.43	0.10
Shape of tassel	-0.35	0.63	-0.03	0.42	-0.18	-0.02
Angle between main axis and lateral branches	-0.68	0.33	0.36	0.24	-0.02	0.15
Number of primary lateral branches	-0.63	0.44	0.17	-0.11	0.39	0.25
Attitude of lateral branches	-0.55	0.43	0.12	0.55	-0.02	0.16
Length of main axis above lowest lateral branch	-0.18	0.15	-0.64	0.54	0.16	-0.27
Length of main axis above highest lateral branch	0.14	0.24	**-0.82**	0.35	-0.04	-0.22
Length of lateral branch	**0.74**	0.24	-0.35	0.23	0.06	0.18
Shape of stem	0.37	0.22	0.17	0.34	0.58	-0.08
Eigen Values	4.98	3.68	3.11	2.71	1.86	1.11
Percent of Variance (%)	23.70	17.53	14.79	12.93	8.85	5.27
Cumulative percentage (%)	23.70	41.23	56.02	68.95	77.80	83.07

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the sixth PC explained the variability 8.85% and 5.27% respectively. No variable has an absolute loading factor value of more than 0.70 in PC5 and PC6. The variables at PC5 and PC6 account for a negligible portion of the variance, indicating these variables do not contribute to the variability in these PCs.

Of the four PCs that show traits that contributed to each PC, only PC1 shows positive values, while the other is negative. this means that traits that contributed to PC1 (the intensity of green colour, anthocyanin coloration of brace roots, and length of lateral branch) show the maximum contribution to the variability in PC1. Meanwhile, traits the intensity of anthocyanin coloration of silk, the length of main axis above highest lateral branch, and degree of zigzag contributed to PC2, PC3, and PC even though they have contributed to variability, their contribution is not optimal. Traits with positive loading factors contributed the most to variability, whereas the negative traits contributed but not optimally (Arif et al., 2023; Saleem et al., 2023).

This study only shows the PCA biplot PC 1 vs. PC 2. Previous research has revealed that PC 1 and 2 have the highest variation and are considered enough to represent all variations (Lee et al., 2020; Ravikumar & Somashekar, 2017). The distribution of genotypes across the vectors in the biplot diagram results in the formation of distinct genotype groups (Khan et al., 2022; Leite et al., 2018). Figure 1 shows the biplot PC1 vs. PC2 for qualitative traits. Han et al (2019) say that the point position in the biplot represented the degree of similarity. The lines that connect the traits to the biplot origin are called trait vectors. The angle between two trait vectors describes their correlation (Maulana et al., 2023). A closer angle shows a closer correlation. Two traits that show an acute angle mean have a positive correlation, an absolute angle has a negative correlation, and a right angle is not correlated.

Figure 1 shows that based on the angle to the UMB trait, the ASCh trait has a strong positive correlation. The AB trait shows a weak negative correlation, and the ABMALB trait does not correlate. The ASCh trait exhibits a higher degree of similarity to the UMB trait than the ACI trait, even though their angles to the UMB trait are nearly identical. This similarity between the two traits is influenced by both the length of their vectors and the cosine of the angle between them. Notably, the ACI trait vector is longer than the ACSh trait vector despite having nearly the same angle.

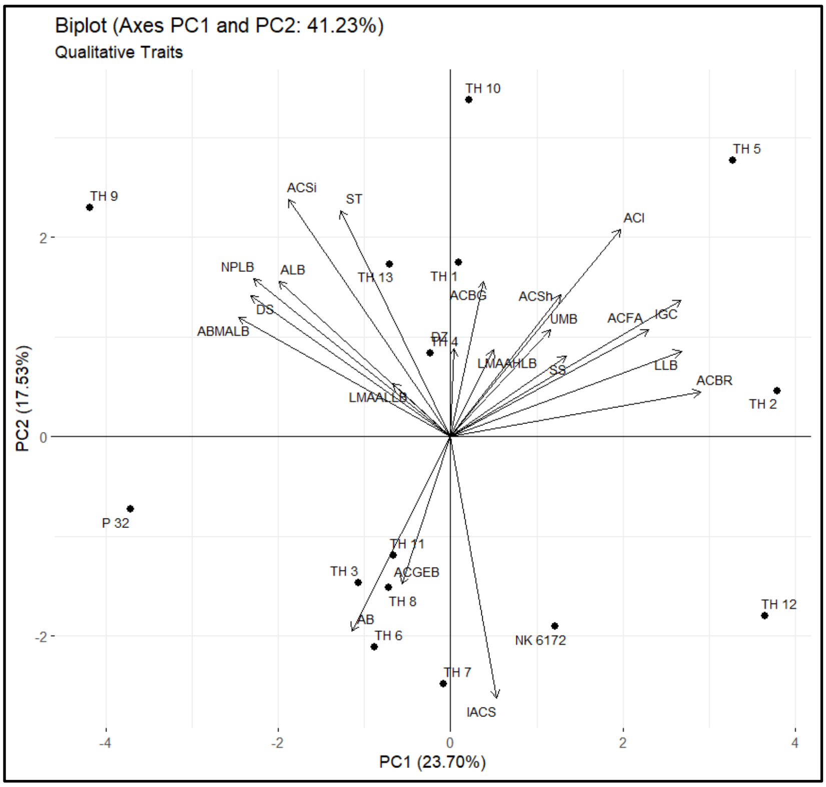

Figure 1. Biplot PCA (PC1 vs PC2) for qualitative traits.

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Genetic variability based on the quantitative trait. The PCA generates the quantitative traits into five PCs with eigenvalues between 1.22 and 5.47 Rojas-Valverde et al. (2020), state that eigenvalues quantify the effectiveness of a factor in capturing the maximum variance from each analyzed variable. The generated PCs represent 84.46% of the total variation. In the other research, Prayudha et al., (2019) generated five PCs for morphology traits and four for agronomy traits in purple-fleshed sweet potato clones. These PCs account for 89.42% and 84.79% of the explained variation, respectively.

In this research on quantitative traits, PC1 covers 32.19% of the variability, influenced by traits such as days to anthesis, days to silk, leaf length, 1000 seed weight, and yield, with loading factor values of 0.90, 0.90, 0.77, -0.77, and -0.77, respectively. PC2, which explains 20.39% of the variation, is impacted by the number of row seeds per ear (loading factor = 0.99) and ear diameter (loading factor = 0.77). Ear height, with a loading factor of 0.75, influences PC3, covering 13.03% of the variability. PC4 explains 13.03% of the variability, driven by the trait ear length (loading factor = 0.81). Lastly, the number of seeds per row, with a loading factor of 0.71, affects PC5, contributing 7.16% to the total variation (Table 3). Although 1000 seed weight and yield affect variability, their contribution is not as high as that of the other traits. Moreover, the 1000 seed weight and yield have negative loading factors, while the others have positive ones. This information aligns with the findings of Gewers et al., (2021) that even though the negative loading factor shows a high contribution, the statistically significant number was fewer than the positive one.

Figure 1 shows the PCA biplot PC 1 vs. PC 2 for quantitative traits, and objectively presents the correlations between these traits. Consistent with the qualitative trait pattern. The Y vector exhibits a positive correlation with both FEW and W 1000, as evidenced by its acute angle to these vectors. In the meantime, the NSR and LA vectors and the Y vector form a straight line. With a cosine value of -1, the straight line shows that Y strongly correlates negatively with NSR and LA. On the other hand, SP and MC form a right angle with the Y vector. Thus, there is no correlation between these traits and Y.MC from a right angle with the Y vector. Thus, there is no correlation between these traits and Y.z

Table 3. Eigenvalues, Variation Explained (%), Cumulative Variance (%), and Loading Factor of Quantitative Trait.

Trait and Component	Principal Component				
	1	2	3	4	5
Days to anthesis	**0.90**	-0.01	-0.07	0.28	-0.17
Days to silk	**0.90**	0.03	0.02	0.25	-0.13
Plant height	-0.36	0.07	0.51	0.50	-0.35
Ear height	-0.31	0.50	**0.75**	-0.06	0.00
Stem diameter	0.44	0.62	-0.44	0.17	0.03
Leaf length	**0.77**	0.03	0.05	0.26	-0.33
Leaf width	0.35	0.50	0.01	-0.48	0.29
Leaf angle	0.59	-0.20	-0.45	0.22	0.39
Moisture content	0.51	0.50	0.52	-0.12	-0.10
Ear length	0.18	-0.36	0.04	**0.81**	0.14
Ear diameter	-0.12	**0.90**	-0.26	0.12	0.05
Number of row seeds per ear	0.19	**0.77**	-0.40	0.11	-0.24
Number of seeds per row	0.29	-0.11	0.42	0.26	**0.71**
1000 seeds weight	**-0.77**	0.12	-0.47	0.01	0.06
Shelling percentage	-0.65	-0.48	-0.25	0.16	-0.25
Fresh ear weight	-0.57	0.61	0.10	0.45	0.16
Yield	**-0.77**	0.34	-0.07	0.47	0.13
Eigen Values	5.47	3.47	2.21	1.99	1.22
Percent of Variance (%)	32.19	20.39	13.03	11.70	7.16
Cumulative percentage (%)	32.19	52.58	65.60	77.30	84.46

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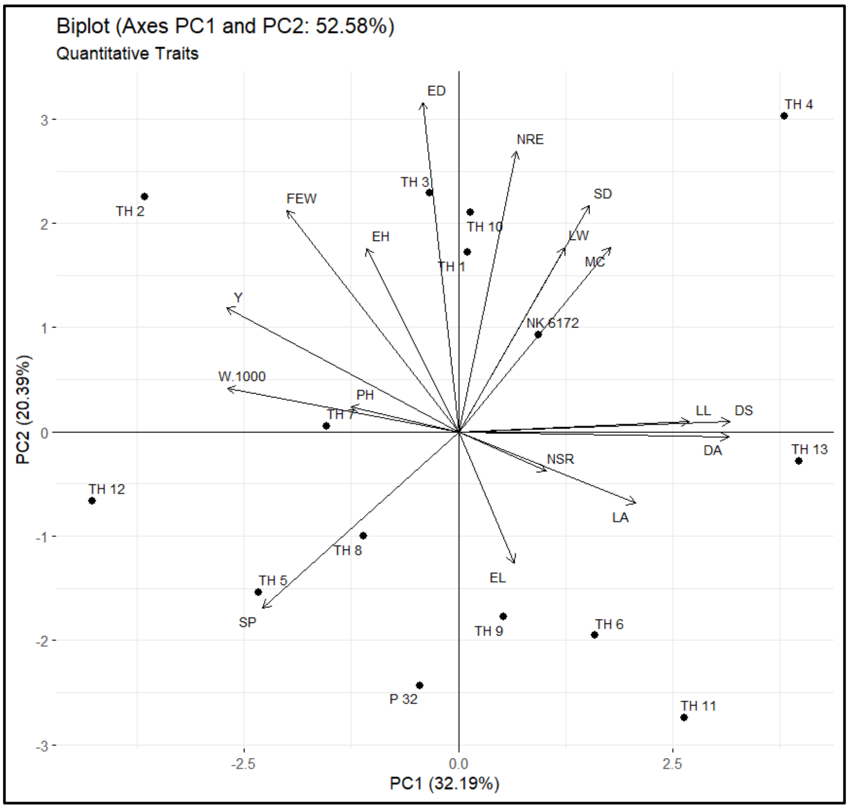

Figure 2. Biplot PCA (PC1 vs PC2) for quantitative traits.

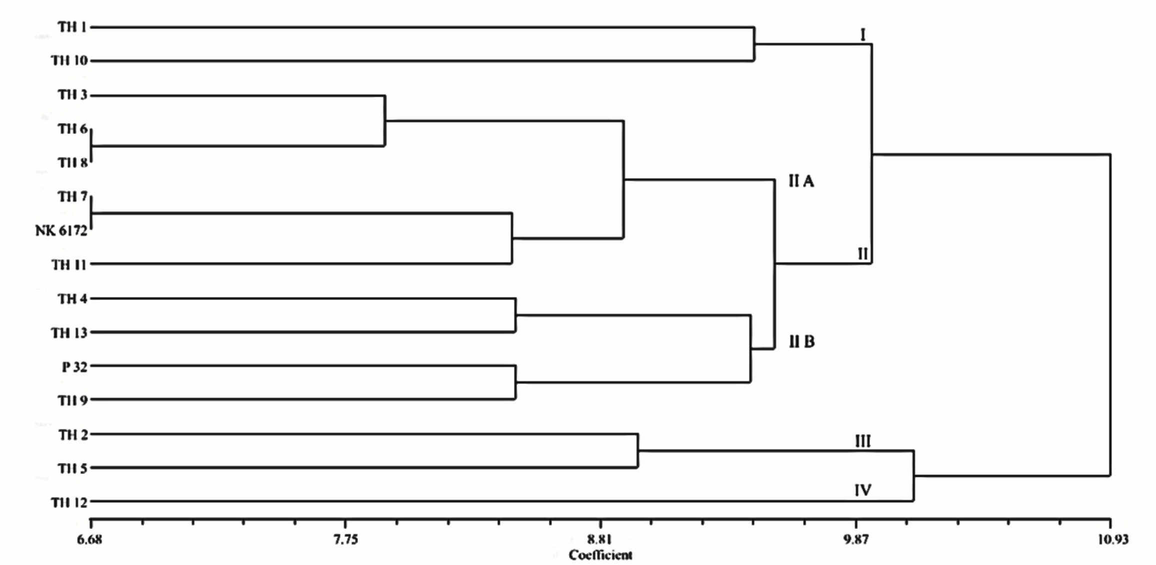

Figure 3. Dendrogram image based on the qualitative trait.

Genetic variability based on the cluster analysis. A cluster analysis based on qualitative and quantitative traits was used to assess the genetic diversity of maize varieties. Based on these traits, the cluster analysis shown as a dendrogram showed that each variety was not as similar as the others (Zhang et al., 2017). This can be seen from the Euclidean Distance in and Figure 4 of the dendrogram. Extensive relationships within genotypes are when the Euclidean distance between two genotypes is greater than 1, demonstrating the lack of a close relationship between the tested genotypes (Torres et al., 2019). The Euclidean value for the qualitative traits dendrogram ranges from 6.68 to 10.93; for quantitative traits, it ranges from 3.4 to 5.08.

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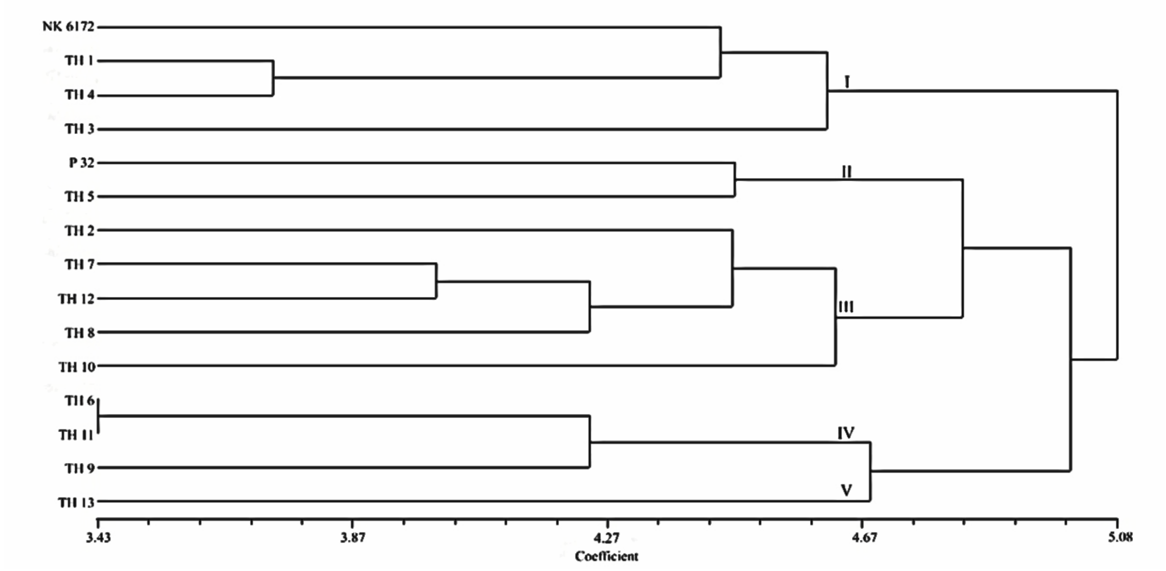

Figure 4. Dendrogram image based on quantitative trait

According to the Euclidean value of qualitative and quantitative traits, this study's maize varieties have a broad variability.

displays a dendrogram image based on qualitative trait. Four clusters of varieties of maize have been identified at Euclidean distance 9.87. Cluster I consists of TH 1 and TH 10. T Cluster II was divided into two subclusters. Five varieties constitute Cluster II A: TH 3, TH 6, TH 8, TH 7, and NK 6172. Furthermore, cluster II B involved TH 4, TH 13, P 32, and TH 9. Cluster III includes TH 2 and TH 5. The rest of the cluster (cluster IV) only contains one variety, i.e., TH 12.

Dendrograms based on quantitative traits appear in Figure 4. This dendrogram classified the maize variety into five clusters at Euclidean distance 4.67. NK 6172, TH 1, and TH 4 varieties constituted cluster I. Cluster II contains TH 3 and P 32. Cluster III is a cluster with the largest number of varieties (five varieties). The varieties are TH 2, TH 7, TH 12, TH 8, and TH 10. Cluster IV consisted of three varieties: TH 6, TH 11, and TH 9. There was one genotype, namely, TH 13, in cluster V.

In genetic variability, genetic distance plays an essential part in plant breeding. Besides genetic variation, genetic distance also plays a vital role in plant breeding. By studying genetic distance, plant breeders can identify sources of trait variation for plant breeding (Juma et al., 2021; Ustari et al., 2023). Genetic distance describes varieties' similarity degrees based on their traits. Two varieties with close genetic distance have a close relationship and high similarities, and vice versa. In dendrogram images, varieties with high similarity degrees are likely found in similar clusters (Metsalu & Vilo, 2015). Figure 1 shows that the highest level of similarity between varieties for qualitative traits was identified between TH 6 and TH 8 and between TH 7 and NK 6172. The varieties TH 6 and TH 11 have been identified as having the highest level of similarity in quantitative traits. The varieties TH 12 for qualitative qualities and TH 13 for quantitative traits exhibit the slightest similarity to the other varieties.

Conclusion

Based on loading factor values more than 0.70, the qualitative traits such as intensity of green colour, anthocyanin coloration of brace roots, length of lateral branch, intensity anthocyanin coloration of silk and degree of zigzag displayed high variability. Quantitative like days to anthesis, days to silk, leaf length, 1000 seeds weight, yield, ear diameter, number of row seeds per ear, ear height, ear length, and number of seeds per row also exhibit high variability. Cluster analysis shows a broad genetic variability on qualitative and quantitative traits demonstrated by Euclidean levels 6.68-10.93 and 3.43-5.08, respectively, and generated the dendrogram that divides genotypes into four main clusters for qualitative and five for quantitative traits.

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Correlation of arbuscular mycorrhizal dosage with level of colonization, nutrient concentration, and photosynthesis pigment of cavendish banana plant

Abstract. The commercial development of Cavendish bananas still faces many obstacles, including the availability of seedlings/suckers at low prices, so technological substitution is needed to stimulate the growth of Cavendish banana suckers in the form of arbuscular mycorrhizal (AM) biological agents. The research aims to determine the correlation between AM doses and colonization levels, tissue nutrient concentrations, and photosynthetic pigments in Cavendish banana seedlings/suckers. It is also a novelty in this research study. The research was carried out in Parepare city at coordinates 3°59'30.204" S; 119°38'42.936" E using four AM doses as independent variables, namely 0 g, 5 g, 10 g, and 15 g pot^{-1}. The variables observed (dependent variables) were the level of colonization, nutrient concentration in the tissue, and photosynthetic pigment content. Using Microsoft Excel software, statistical tests, regression, and correlation analyses were conducted to see the relationship between treatment (independent variable) and observed parameters (dependent variable). The research showed that AM dosage was positively correlated with the level of root colonization, the concentration of N, P, and K elements in the tissue, and the photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids). A dose AM of 15 g pot^{-1} gave a better effect on Cavendish banana suckers, which can be recommended for the development of Cavendish banana seedlings.

Keywords: Chlorophyll · Endomycorrhiza · Infection · Nutrients · Suckers

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Introduction

Bananas, as a horticultural crop, are ranked fourth as the most essential food commodity in the world after rice, corn, and wheat (Awa et al., 2024); (Hariyanto et al., 2021); (Tan, 2022). The types of bananas in Indonesia are very diverse, including the Cavendish banana (*Musa acuminata* L.) discovered by William Cavendish (Hariyanto et al., 2021); (Hastuti et al., 2019). This banana comes from Mauritius (East Africa) and was first planted at Chatsworth House (England) in the 1830s with Joseph Paxton. In 1900, Cavendish banana cultivation became increasingly widespread in various places in the world (Bragard et al., 2021); (Justine et al., 2022); (Perrier et al., 2019).

Cavendish bananas have high economic value, especially as an export commodity (Acevedo et al., 2021); (Alho et al., 2021); (Veliz et al., 2022). In 2020, Cavendish bananas were exported to China, Hong Kong, Malaysia, the United Arab Emirates, and Pakistan, with a contribution of 6% to total fresh fruit exports from Indonesia (Nola et al., 2022; Sukmaya et al., 2022). According to Afzal et al. (2022) and Phillips et al. (2021), Cavendish bananas are mostly consumed as fresh fruit and as raw material for making banana flour (Lorenzo et al., 2024; Maseko et al., 2024; Sawant et al., 2023).

Some of the nutritional content found in 100 g of Cavendish bananas is an energy value of 110.15 kcal; Fiber 2.3%; Calcium 12.5 mg; Magnesium 41.5 mg; Iron 0.9 mg; Copper 0.3 mg; Zinc 0.2 mg; Manganese 0.1 mg; Vitamin C 18.5 mg; Protein 0.3 mg; Phosphorus 59.1 mg; Carbohydrates 26.2%; β-carotene 21–290 μg; potassium 400 mg, apart from that also found vitamin A, vitamin B6, riboflavin, folate, fathothanic acid and niacin (Huang et al., 2024; Mustakin et al., 2023; Phillips et al., 2021; Siriwardana et al., 2019; Zein et al., 2023).

Commercial development of Cavendish bananas still faces many obstacles, including providing superior sucker in large quantities at affordable prices. Cavendish banana plants can be cultivated conventionally by separating suckers from clumps or tubers (corm). Still, the number of fully grown banana suckers obtained is only 2 – 3 suckers per parent or 5 – 10 per year (Blende & Kurien, 2015); (Sorn et al., 2024). Another method that can be used to cultivate Cavendish bananas is by dividing the tubers (corm) according to the number of adventitious/axillary buds and plant roots, which is also commonly used as a biological agent (Sapheera et al., 2024; (Tumuhimbise & Talengera, 2018); (Wahab et al., 2023); (Weng et al., 2022). Farmers can easily use this method to get more and faster sucker of bananas. However, this method needs to be supported with technology that can stimulate the root growth of the Cavendish banana. One technology that can be applied is using biological agents.

Mycorrhiza is a form of association between fungi (Figueiredo et al., 2021; Wahab et al., 2023). Using biological agents such as arbuscular mycorrhizal (AM) which colonize plant roots can increase the growth of plant seedling/sucker roots in large quantities to increase nutrient absorption (Gough et al., 2020; Khan et al., 2022); Wahab et al., 2023). Indrawati & Suswati (2019) and Castillo et al. (2016) stated that the application of AM *Glomus* sp. and *Acauluspora* sp. had a positive effect on increasing the growth of the roots of Barangan banana seedlings/sucker cultured in vitro, although the wet root weight did not have a statistically significant effect. In Musa paradisiaca (local variety of Ardhapur), the percentage of mycorrhizal colonization reached 60 to 70%, and the mycorrhizal structures colonizing the root tissue were round and elongated vesicles and arbuscules (Wankhede & Mulani, 2023). These three studies have not stated what dose of AM can have a positive effect on AM activity and the physiological supporting components of Cavendish banana seedlings/sucker. This study aims to determine the correlation of AM dose with colonization level, nutrient concentration in tissue, and photosynthetic pigments in Cavendish banana seedlings/shoots. However, in this study, the hypothesis that can be raised is that there is a positive correlation between AM dose and dependent variables (colonization level, nutrient concentration in tissue, and photosynthetic pigments), which is also new in this study.

Materials and Methods

This research was conducted in the research area of Agroplastid Farm, Bukit Harapan District, Soreang Regency, Parepare City, South Sulawesi, at an altitude of 37 m above sea level with coordinates 3°59'30 "S and 119°38'43 "E.

Preparation of planting media. The sand and husk charcoal are cleaned and sifted before steaming for 8 hours. After cooling, the two-planting media are mixed in a 1:1 ratio and placed in a labeled culture pot.

Figure 1. Preparation of materials for propagating cavendish bananas using tubers.

Preparation of materials for propagating Cavendish banana. Cavendish banana axillary shoots were obtained from banana tubers by dividing the tuber 10 x 10 cm according to the number of axillary shoots. The axillary buds that have been separated are cleaned using running water and then soaked in a disinfectant solution for ± 2 minutes to kill pathogenic microorganisms.

Preparing the AM doses. The AM propagules are weighted according to the treatment. One gram of AM propagule contains carrier media (sand, zeolite, and biochar), root pieces of the host plant, and 80 – 100 units of spores.

The study used four AM doses as treatment (independent variable): without AM application (control), dose 5 g, 10 g, and 15 g pot^{-1} culture. Meanwhile, the variables observed were the level of AM colonization, the concentration of N, P, and K in the tissue, and the content of photosynthetic pigments (dependent variable) after the plants were 30 days old after being incubated with AM.

The level of AM colonization and analysis of AM-colonization roots were carried out at the Environmental Technology and Security (ETS) Section Laboratory, SEAMEO BIOTROP, Bogor. The technique for observing mycorrhizal colonization is root staining, which is done by selecting fine, fresh roots from the roots of sample plants. The roots were placed in a tube containing FAA (formaldehyde, alcohol, acetic acid) solution for 24 hours. The FAA solution is discarded, and the roots are washed until clean. Next, the roots are soaked again in 10% KOH solution for 24 hours. The KOH solution is then discarded, and the roots are washed clean. Next, the roots are soaked in a hot H_2O_2 solution for 24 hours and washed thoroughly. The roots that have been washed thoroughly are soaked in a 2% HCl solution for 24 hours. The HCl solution was discarded, and the fine roots were washed with running water. Next, the roots were soaked in 0.05% trypan blue solution for 24 hours. Then, one root sample with a length of 1 cm was taken from the colored roots and arranged on a glass slide. Root pieces on slides were observed at each angle. The percentage of root infection was calculated using the following formula (Brundrett et al., 1984).

$$\text{Root Colonization}(\%) = \frac{\sum \text{roots area colonization}}{\sum \text{roots area observed}} \times 100$$

The percentage of colonized roots was determined based on the criteria of Rajapakse & Miller (1992) as follows:

- <5% = very low
- 6 – 25% = low
- 26 – 50% = medium
- 51 – 75% = high
- >75% = very high

The N, P, and K concentrations in root, stem, and leaf tissue were analyzed at The Soil Chemistry and Fertility Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Hasanudin. The N concentration in the tissue was analyzed using the Kjeldhal method, while the P and K concentrations were analyzed using the wet ashing method with HNO_3 and $HClO_4$.

The total content of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in leaves was analyzed in the Water Productivity and Quality Laboratory, Faculty of Marine Affairs and Fisheries, Hasanuddin University, using the spectrophotometer method with acetone as the solvent.

Data from laboratory tests were analyzed statistically using Microsoft Excel software. Statistical regression and correlation analysis tests were conducted to see the relationship between the treatment (independent variable) and the observed parameters (dependent variable).

Results and Discussion

The results of the analysis of the level of colonization in the root tissue of Cavendish banana seedlings based on the criteria of Rajapakse & Miller (1992) showed that the roots of Cavendish banana

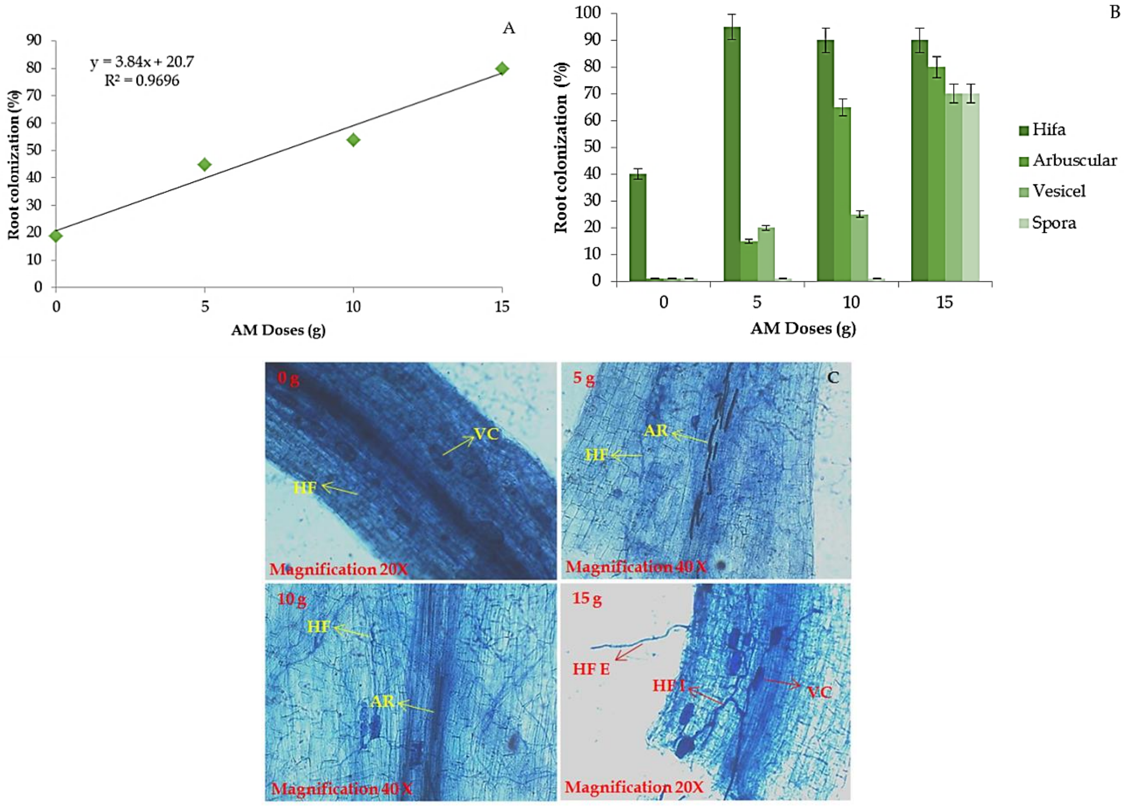

Figure 2. Relationship between AM dose and root colonization at 60 DAT (A), percentage of AM organelle colonization is the average value ± SE from 10 root samples examined; Vertical bars show the Standard Error (SE) value at the 5% level (B); and cross sections of roots of cavendish banana seedlings/sucker infected with AM at 30 DAP with 20 and 40 times magnification (HF, Hyphae; HFE, External Hyphae; HFI, Internal Hyphae; VC, Vesicles; AR, Arbuscula; SP, Spores) (C).

seedlings that were applied with AM at different doses showed AM colonization activity which was in the category of low (6% - 25%) to very high (>75%), and The results of regression and correlation analysis showed that the AM dose treatment had a very strong correlation (R=0.9847) with 97% of the variation in root colonization explained by the AM dose (R^2=0.9696) (Figure 2A). Application of doses of 5 g, 10 g, and 15 g pot^{-1} showed an increase in root colonization activity of 57.78%, 64.81%, and 76.25%, respectively, carried out by AM structures in the form of hyphae, arbuscular, cysts, and spores mainly when AM was applied at a dose of 15 g pot^{-1} (Figures 2B and 2C). The results of correlation analysis in Figure 2A and infection activity in Figures 2B and 2C are in line with the previous observations by Rashad et al. (2021) and Lin et al. (2021) that showed the level of AM (*Gigaspora macrocarpum*) infection reached 69-71% in the root tissue of banana plantlets where AM was applied to a planting medium. Meanwhile, treatment without AM showed the lowest results, namely 0%. This phenomenon occurs because the compatibility between the type of AM and the host plant (Cavendish banana) is not an inhibiting factor in colonizing Cavendish banana roots by AM hyphae. This colonization benefits both of them, namely that AM gets energy from carbohydrates in the form of glucose (simple sugar) from Cavendish banana. Cavendish bananas can increase nutrient absorption with the help of AM hyphae. Previous studies show that banana plants, as a highly mycotropic species, are plants that have a high level of dependence on AM, namely 37%-46% when the plant does not experience stress and increases to 59%-74% when the plant experiences stress (Avila et al. 2022; Dwiyani et al. 2024; Rashad et al. 2021; Ramirez-Silva et al. 2022).

The statistical analysis of nutrient concentrations in Cavendish banana seedling tissue showed a different phenomenon. The mycorrhizal dose factor has a very high/strong correlation (R=0.9502) for nitrogen (N) nutrients and a high/strong correlation (R=0.8186) for

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Phosphorus (P) and Potassium (K) nutrients (R=0.8438). Mycorrhizal dose affected 90% of N concentration (R^2=0.9029), 67% concentration of P (R^2=0.6701), and 71% of K (R^2=0.7121) in Cavendish banana seedling tissue (Figures 3A, 3C and 3E). Applying AM doses of 5 g, 10 g, and 15 g pot^{-1} can increase the N concentration in cavendish banana seedling tissue by 8.59%, 9.46%, and 20.17%, respectively (Figure 3A). The highest N concentration was found in leaf tissue, followed by stem and root tissue, especially in the AM treatment dose of 15 g pot^{-1} (Figure 3B).

Doses of 5 g, 10 g, and 15 g pot^{-1} could increase the P concentration in Cavendish banana seedling tissue; respectively, the P concentration increased by 4.55%, 19.23%, and 14.29% (Figure 3C). The highest P concentrations were found in root and leaf tissue in all AM dose treatments (Figure 3D).

The concentration of element K in Cavendish banana seedling tissue increased by 24.12%, 25.33%, and 26.88% with the application of AM doses of 5 g, 10 g, and 15 g pot^{-1} (Figure 3E), and this element accumulates more in plants roots and stems (Figure 3F).

The phenomenon in Figures 3A and 3B is possible that AM performance can increase the N element in the tissue by increasing the number of hyphal structures (Figure 2). AM hyphae can intensify N-fixing microorganisms to provide a source of N in the soil (Dellagi et al., 2020; Ghorui et al., 2024; Jansa et al., 2019). AM reduces N nutrient loss through mineralization by abundant N-fixing

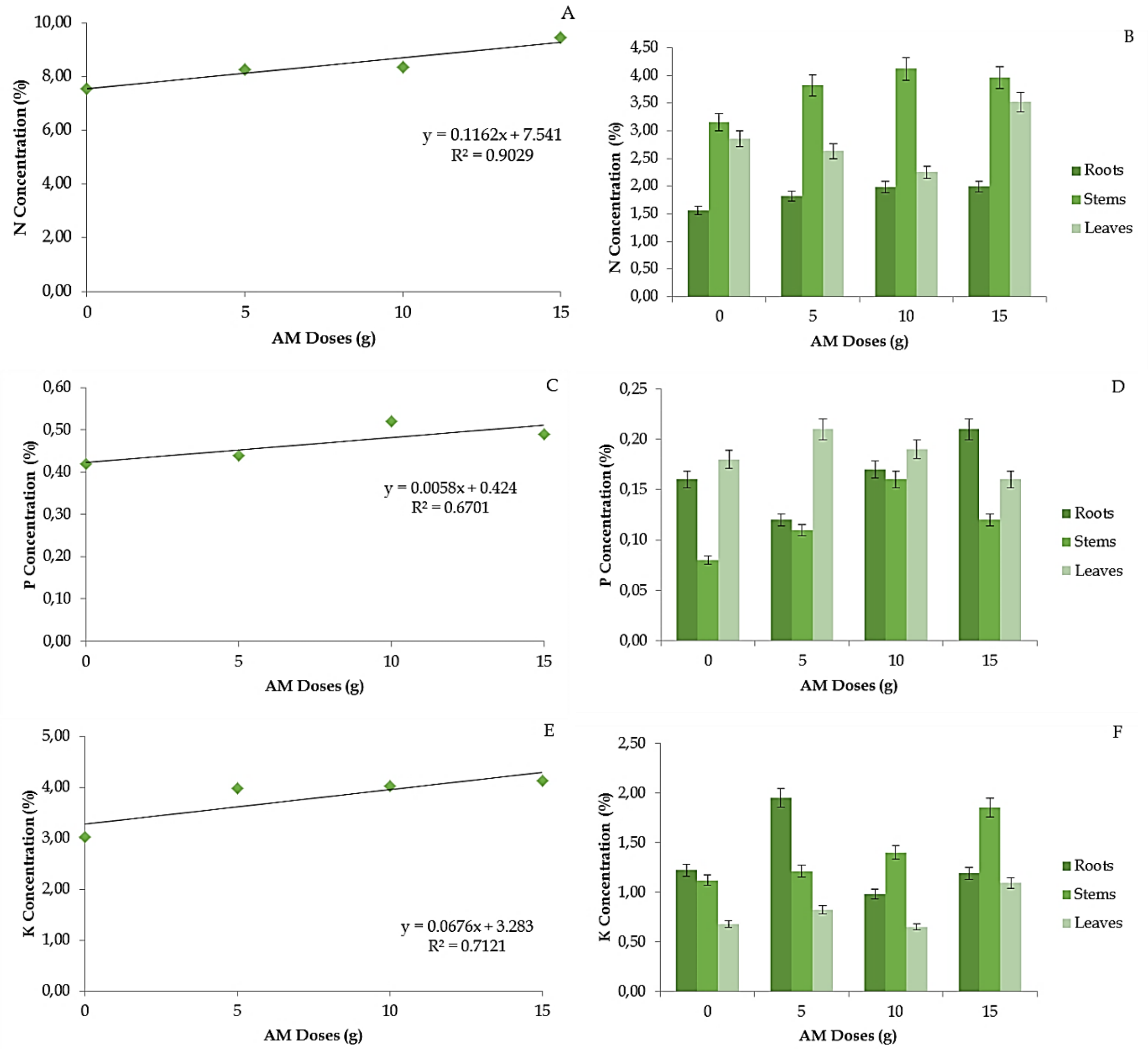

Figure 3. Relationship between AM dose and nutrient concentration in cavendish banana seedling/sucker tissue at 90 DAP (A, C, E). Nutrient concentration values are the average calues ± SE of the 12 samples examined. Vertical bars show the Standard Error (SE) value at the 5% Level (B, D, F).

bacteria due to improved soil structure by AM (Martin & van der Heijden, 2024; Santoyo et al., 2021). This results from the external hyphae of AM, which provide C as an energy source for N-fixing bacteria (Hawkins et al., 2023; Hoosein et al., 2023; Wang et al., 2024). The compatibility of AM with the roots of Cavendish banana seedlings allows the roots to expand the absorption of nutrients by the roots, so the growth of Cavendish banana seedlings can be promoted. Previous studies stated that the N is essential to all living cells (Grzyb et al. 2021; Govindasamy et al. 2023; Zayed et al. 2023)). In plants, N functions as the main constituent component of amino acids, proteins, hormones, chlorophyll a and b, vitamins, and enzymes, which are essential for plant life (Baslam et al., 2021; Fathi, 2022; Nieder & Benbi, 2022). Nitrogen makes up 40%-50% of the dry weight of cell protoplasm, which is the physical basis of living cells (Alharbi et al., 2022; Kerru et al., 2020; Parashar & Jain, 2020). Therefore, N is needed in large quantities to support the plant growth process (Anas et al., 2020; Leghari et al., 2016).

The increase in P concentration in Cavendish banana seedling tissue shown in Figures 3C and 3D is possibly caused by the activity of AM hyphae, which secrete phosphatase enzymes to dissolve P bound in the soil so that it can be available to plants. In plant cells, P is an essential element that plays a role in physiological and biochemical processes, especially in producing, storing, and transferring energy in the form of Adenosine diphosphate (ADP) and Adenosine triphosphate (ATP) (Javed et al., 2022); (Khan et al., 2023); (Xiao et al., 2024). ATP is necessary for plant growth because cell division and increasing the number of cells require energy in the form of ATP (Althaher & Alwahsh, 2023); (Braun, 2020). The research results of Soumare et al. (2021) and Wahome et al. (2023) showed that giving AM gave the best growth response to bananas from in vitro culture after acclimatization. Etesami et al. (2021); Kuila & Ghosh. (2022); Zhao et al. (2024) added that plants infected with AM could produce high P uptake. External hyphae from AM support the increase in P concentration by mycorrhizal infected plants through an increase in surface area, which is more effective in absorbing P elements from the soil to plant roots (Begum et al., 2019; Kuila & Ghosh, 2022; Wahab et al., 2023). Element P is an easily mobile plant nutrient (Doydora et al., 2020; Ibrahim et al., 2022; Khan et al., 2023; Pang et al., 2024). If there is a P deficiency, the P translocation originates from the soil solution, and the older leaves (translocation) go to the roots to be used in root formation (Dixon et al., 2020; Nadeem et al., 2022; Solangi et al., 2023; Zhao et al., 2021). Therefore, P accumulation will increase in the roots during the growth period (Bagyaraj et al., 2015; Chen et al., 2023; Liu, 2021). Several research results show that banana seedlings in symbiosis with AM have a higher growth response and P absorption compared to seedlings without AM. The effectiveness of AM infection inoculated on banana seedlings reaches 23.7%–71.7%, but each type of AM is effective—different infections for each banana seedling (Das & Sarkar, 2024; Etesami et al., 2021; Ferrol et al., 2019; Lin et al., 2021).

The K element in Cavendish seedling/sucker tissue has increased in concentration (Figures 3E and 3F); the increase in K concentration may be the effect of the enhanced P concentration in Cavendish banana seedling tissue. Several previous studies have proposed several hypotheses that increased K accumulation in mycorrhizal plants may be a partial consequence of increased P uptake (Alam et al., 2023; Chandrasekher & Ray, 2020; Luan et al., 2017) and the second hypothesis proposes that K becomes one of the primary counterions of polyphosphates that form K ions (Hashem et al., 2018; Micheluz et al., 2022; Pipatpolkai et al., 2021). Wilkes. (2021) and Kaba et al. (2021) shows that K accumulation is found in spores, hyphae, arbuscular, and AM vesicles and shows that high K concentrations are found in roots. Han et al. (2023) and Mdrid-Delgado et al. (2021) confirmed that the abundance and distribution of K are correlated with the abundance of P in spores at tissue root plants. In AM fungi, P is present mainly as polyphosphate (Petriglieri et al., 2022; Tarayre et al., 2016; Wahab et al., 2023), and it is thought that K acts as a counterion to P in polyphosphate (Christ et al., 2020; Herrmann et al., 2023; Muller et al., 2019) thereby stabilizing the molecule. Calcium polyphosphate deposits previously reported for the AM structure are now believed to be an artifact caused by chemical fixation (Chen et al., 2024; Hazzoumi et al., 2022). The spatial distribution of P and K in spores is closely related (Ji et al., 2022; Xue et al., 2017). No other elements are associated with P in this way, indicating a specific interaction between K and P (Luan et al., 2017; Xie et al., 2021). In addition, the K content tends to increase in spores when the P content increases. Elhindi et al. (2017); Srivastava et al. (2018); Yang et al. (2017) found high levels of P and K in AM vacuoles; their abundance appears to be interrelated. This may be due to the polyphosphate transport mechanism in the vacuolar system.

Mycorrhizal colonization on plant roots can expand the area of root absorption by the presence of external hyphae that grow and develop through root hairs. Hyphae involved in the root system can extend the root absorption range to 80 -100 mm compared to plants without AM (only 1 - 2 mm) and increase the nutrient absorption rate six times faster (He et al., 2020; Hou et al., 2021). Plants infected with AM can absorb higher levels of P fertilizer (10-27%) than plants without mycorrhizae (0.4-13%). Mycorrhizal fungi can replace approximately 27-50% of the use of phosphate, 40-50% of nitrogen and 20-25% of potassium (Prayogo et al., 2021; Rui et al., 2022; Yuwati et al., 2020). Chen et al. (2018) and Tran et al. (2020) explained that mycorrhizal fungi can help absorb nutrients and prevent leaching of nutrients.

Statistical tests on the content of photosynthetic pigments in the leaf tissue of Cavendish banana seedlings showed different phenomena. The AM dosage factor has a high/strong correlation with chlorophyll a pigment (R=0.7105) and a very high/strong correlation with chlorophyll b pigment (R=0.9190) and carotenoids (R=0.9505). Furthermore, the dose of AM affected 50% of the chlorophyll a content (R^2=0.5048), 84% chlorophyll b content (R^2=0.8445), and 90% carotenoid content (R^2=0.9035) in the leaf tissue of Cavendish banana seedlings/suckers (Figures 4A, 4B and 4C). The results of photosynthesis pigment analysis also show that chlorophyll a pigment ($C_{55}H_{72}O_5N_4Mg$) is more dominant than chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) and carotenoids/carotene ($C_{40}H_{56}$) (Figure 4D).

The increase in photosynthetic pigments, as shown in Figures 4A, 4B, and 4C, may be related to the concentration of N in the leaves, the absorption of which is assisted by AM hyphae, thereby accelerating the absorption of light intensity and increasing the photosynthetic capacity of the leaves, which ultimately produces carbohydrates to support AM performance.

According to Begum et al. (2019); Delaeter et al. (2024); Saboor et al. (2021a); Wahab et al. (2023) the symbiosis of AM with roots can increase the chlorophyll content of plants in soil that lacks nutrients, including nitrogen (N). Several research results show that the application of AM (*Glomus mosseae*) can increase the chlorophyll content in *Geranium* (Amiri et al., 2017), *Xanthium italicum* (Shi et al., 2020) and *Zea mays* (Saboor et al., 2021b). Furthermore, Balestrini et al. (2020) and Mathur et al. (2021) explained that the presence of AM will help protect the photosystem II process in the light phase of photosynthesis, which plays a vital role in water absorption and nutrients. However, according to Bell et al. (2024) and Wahab et al. (2023), factors that reduce the photosynthetic capacity of plants will affect the function of AM because fungi in symbiosis are very dependent on the carbon produced by their host plants. Bell et al. (2024); Delaeter et al. (2024); Salmeron-santiago et al. (2022) suggest that AM receive carbohydrates in the form of simple hexose sugars ($C_6H_{12}O_6$) (examples of hexose sugars are glucose and fructose) from the host plant, around 12-27% or 1/3 of the total carbon produced by photosynthesis, and this causes changes in the distribution of photosynthesis—plants to other organs.

The photosynthetic pigment analysis results are dominated by chlorophyll a (Figure 4D), possibly because the leaves of Cavendish banana seedlings are still relatively young, so chlorophyll formation is incomplete. Ebrahimi et al. (2023); Gao et al. (2024); Mulay & Kokate (2019) explain that chlorophyll has not yet formed completely in new and young leaves but is still in the form of protochlorophyll (Protochlorophyll is a green pigment containing magnesium and found in etiolated leaves and seedlings grown in the dark). However, after the transformation of protochlorophyll into chlorophyll, the leaves will become green. Similar results have also been reported for the leaves of *Mangifera indica* (Niaz et al., 2024), *Eleutherine palmifolia* (Ekawati & Saputri, 2022), *Codiaeum variegatum* (Falcioni et al., 2023), *Musa Paradisiaca* (Saha & Zude-Sasse, 2022) and *Syzygium polyanthum* (Adriano et al., 2021). Zhu et al. (2024) and Zhao et al. (2020) stated that apart from genetic factors, chlorophyll formation is also influenced by the quality and quantity of light and Mg^{2+} nutrition as a form and catalyst in chlorophyll synthesis. In plants, about 5–35% of Mg is detected in chloroplasts, and Mg is the central element of the tetrapyrrole ring in chlorophyll (de Bang et al., 2021; Kan et al., 2022). All higher plants contain chlorophyll a and chlorophyll b. Chlorophyll a makes up 75% of the total chlorophyll (Juhaeti et al., 2020); (Veazie et al., 2020) and the re-chlorophyll formation is also influenced by the quality and quantity of light and Mg^{2+} nutrition as a form and catalyst in chlorophyll synthesis.

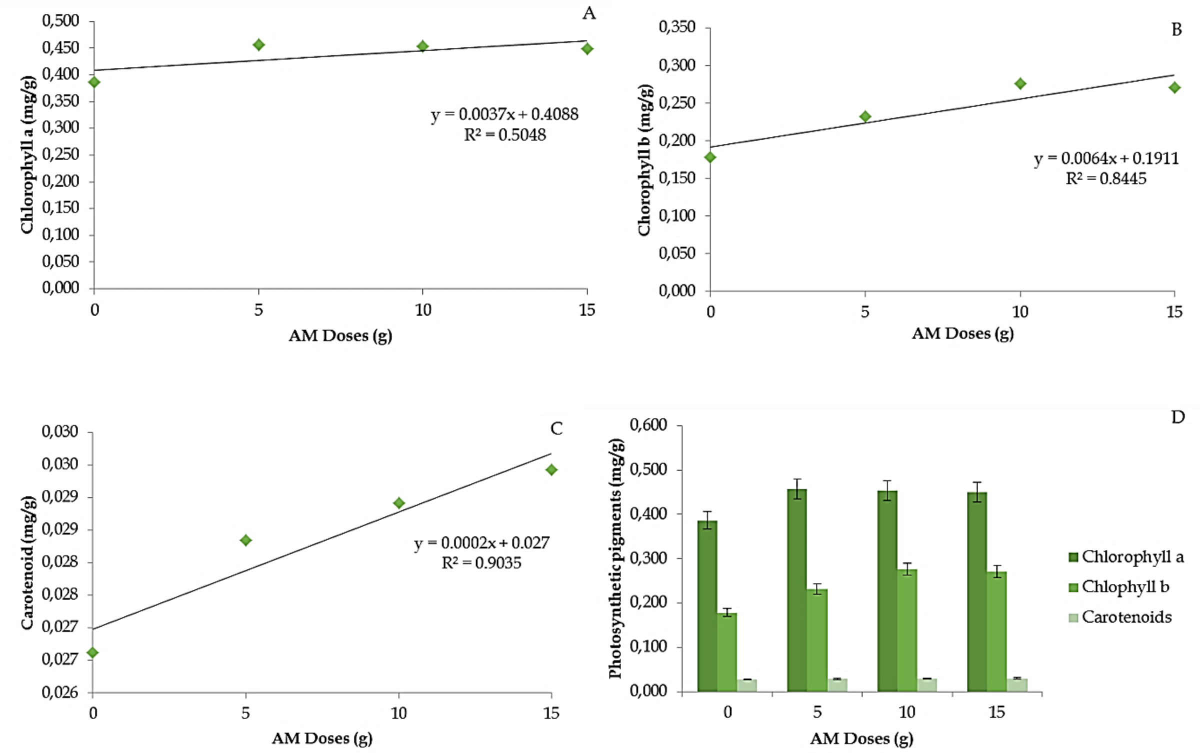

Figure 4. Relationship between AM dose and photosynthetic pigment content in cavendish banana seedling/sucker tissue at 90 DAP (A, B, C). Photosynthetic pigment content is the average value ± SE of the 12 leaf sections examined. Vertical bars show the Standard Error (SE) value at the 5% level (D).

In plants, about 5–35% of Mg is detected in chloroplasts, and Mg is the central element of the tetrapyrrole ring in chlorophyll (de Bang et al., 2021); (Kan et al., 2022) constructing 25% makes up chlorophyll b and other pigments. The chlorophyll content in green plants is around 1% of dry weight (Arshad et al., 2023; Veazie et al., 2020).

Conclusion

The AM dosage is positively correlated with the level of root colonization, the concentration of N, P, and K elements in the tissue, and the content of chlorophyll a, chlorophyll b, and carotenoid pigments as a means of photosynthesis. This has a better effect on the growth of Cavendish banana seedlings/shoots, especially at an AM dosage of 15 g pot^{-1}, so it can be recommended for plant breeders in the propagation of Cavendish banana seedlings.

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Comparative study of rice morphological and physiological characteristics grown under organic and inorganic farming

Abstract. Organic farming practices have shown a potential to improve rice yields, aside from the popular conventional ones. This study aimed to comparatively analyze the morpho-physiological characteristics of rice plants grown under organic and inorganic farming. This research was conducted at the Polinela Organic Farm experimental station (5°21'10"S 105°13'43"E, 114 m sea above level), from February to April 2023 using a completely randomized design. The results showed that rice grown organically exhibited a notably higher chlorophyll index and actual water use efficiency compared to those grown inorganically. Conversely, inorganic farming resulted in a greater number of tillers than organic farming. However, there were no significant differences between the two systems regarding CO_2 efficiency and plant height. An interesting fact is the improved water use efficiency by organic farming helps rice plants to achieve similar growth performance while requiring less water.

Keywords: Chlorophyll index · Conventional farming · CO_2 efficiency · Morpho-physiological characteristics

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Introduction

Rice is a staple food globally, meeting over 21% of human caloric needs and accounting for 76% of caloric intake in Southeast Asia. The cultivation practices for rice significantly influence the crop's quality, safety, and nutritional value. However, the sustainability of these practices is increasingly questioned as organic farming methods are proposed as environmentally friendly alternatives (Mohidem *et al.*, 2022). The critical challenge in rice production lies in balancing productivity with environmental sustainability. While organic farming is touted for its potential to improve soil health, its impact on rice's physiological characteristics, particularly water efficiency and plant health during crucial growth stages, still needs to be explored. This study aims to address this gap by examining how different cultivation methods, mainly organic and conventional practices, affect the morpho-physiological characteristics of rice plants (Chiou *et al.*, 2023). Organic rice cultivation emerges as a potential solution, offering enhanced soil physical properties and improved water use efficiency (WUE) through practices like cover crop planting. This method aligns with sustainable agriculture principles, providing an environmentally friendly alternative for food production (Wacławowicz & Giemza, 2023). However, while organic cultivation may not significantly boost productivity, its profound impact on the natural environment is noteworthy.

As the global population expands and fertile land resources decrease, the strain on food security intensifies. The choice of rice variety directly influences its quality and yield, which is a critical consideration in addressing these challenges (Long *et al.*, 2023). The physical quality of rice plants during the vegetative phase is essential, influencing WUE and productive tiller count (Venkatesan *et al.*, 2023). Challenges persist in managing limited water resources, with disputes over distribution complicating sustainable water management. Organic cultivation patterns and strategic irrigation arrangements demonstrate the potential to optimize rice productivity by enhancing photosynthesis rates during the critical stages of the growth period (Liu *et al.*, 2023). The resultant neglect of water distribution infrastructure is a debatable issue, affecting the technical aspects of plant cultivation, especially during the vegetative phase (Wudil *et al.*, 2023). Despite these challenges, more research is still needed to focus on the morpho-physiological characteristics of the vegetative phase in rice varieties geared towards efficient water use. Consequently, this study aims to undertake a comparative analysis of the morpho-physiological traits of rice plants, considering their cultivation locations and the impact of enhanced WUE achieved through organic farming practices. Improving WUE is expected to lead to better growth and yield outcomes by optimizing water utilization, which influences key morpho-physiological traits such as plant height, number of tillers, and chlorophyll content.

Materials and Methods

This research was conducted at Polinela Organic Farm experimental garden, Politeknik Negeri Lampung, Bandar Lampung, Lampung, Indonesia (5°21'10"S 105°13'43"E, 114 m sea above level), from February to April 2023. A population of 72 rice plants was grown in a tropical greenhouse using organic and inorganic methods. Straws from previous rice plants were allowed to decompose in the soil for four weeks before being utilized in the organic cultivation method. The straw was treated with natural decomposition agents before being incorporated into the soil to accelerate the decomposition process. In the inorganic cultivation method, rigorous soil preparation was undertaken to ensure the absence of any pre-decomposed organic compounds. This process involved thoroughly cleaning and preparing land to remove residual organic matter before applying chemical fertilizers.

The vegetative phase observation focused on two distinct cultivation methods: organic and inorganic. Straws from previous rice plants were employed in the organic method by immersing them in the soil to decompose. Meanwhile, inorganic cultivation employed chemical fertilizers (Dose per plant: 4 g N, 3 g SP36, and 2 g KCl), manual weeding, and pest and disease control when necessary.

Procedure. The research was conducted using a Completely Randomized Design (CRD) with one factor, consisting of two treatment levels: organic and inorganic cultivation. The experiment had eight replications, resulting in 16 experimental units. Each replication consisted of nine pots with rice plants, resulting in 72 rice plants overall. The criterion for selection included the absence of pest and disease attacks, ensuring the chosen rice plants exhibited normal and healthy growth. Utilizing a completely randomized design, the selection aimed to maintain an unbiased representation across both cultivation methods. This study selected rice plants 45 days after planting, typically reaching a height of approximately ±70 cm with 12-17 tillers, based on a standardized sampling method. The experimental plots were divided into six sections, with 2-3 plants being randomly selected from each section. Only plants that were free from pests and diseases were included in the sample to ensure the accuracy of the observed morphological and physiological characteristics (Yassi *et al.*, 2023).

Observation variables were measured in this research. Plant height was measured from the plant stem's base to the flag leaf's tip using a ruler expressed in cm units. The number of tillers is calculated by counting the number of stems in one clump of rice plants. Meanwhile, Soil Plant Analysis Development (SPAD) was administered to measure the chlorophyll content (Zhang et al., 2023). Furthermore, physiological characteristics of rice were observed using a portable photosynthesis system Li-6800XT (Jia et al., 2023), Licor Inc., Lincoln, NE, USA on a sunny day at 9:00 am (Yang et al., 2022), 16 March 2023 with three replications (triplo) that were carried out automatically by the Li-6800XT. The actual water use efficiency (WUE) was measured using a Licor photosynthesis system, which calculates WUE as the ratio of the net photosynthesis rate (A) to the transpiration rate (E). The system expresses WUE in µmol CO_2 mmol H_2O^{-1}, allowing for precise quantification of the plant's water use efficiency under different environmental conditions. Carbon dioxide use efficiency (CO_2) was also determined using the same Licor system. The measurement involves analyzing the CO_2 concentration in the air entering and exiting the leaf chamber and calculating the difference to determine the net CO_2 assimilation. The system reports CO_2 use efficiency as CO_2 mmol H_2O^{-1}, reflecting the plant's ability to utilize available carbon dioxide efficiently. The Licor system uses an infrared gas analyzer (IRGA) to measure the concentration of CO_2 in the air stream. The CO_2 concentration is measured before and after the air passes over the leaf surface, and the difference is used to calculate the rate of photosynthesis. This precise measurement allows for an accurate assessment of both WUE and CO_2 use efficiency.

Data analysis. The collected data was then subjected to a least significant difference (LSD) test at α 5% using the Statistical Tool for Agricultural Research (STAR) version 2.0.1. (IRRI, Los Banos Philippines, 2013).

Results and Discussion

Comparative analysis of rice morphological characteristics**.** Plant height is a crucial growth parameter in rice plants as it dictates traits influencing yield and rice grain production (Wu et al., 2022). The characteristics of rice plants, particularly their height, are influenced by genetically controlled elements, predominantly determined by the genetic composition of the genotype, which is dependent on factors such as the number of internodes and internode length. (Jahan, 2020). In turn, the number of tillers per plant governs the count of panicles, a pivotal component of grain yield. The capacity of tiller production often determines the potential yield of rice cultivation. Higher tiller counts in rice plants may lead to disparities in assimilate and nutrient mobilization between tillers, resulting in variations in grain development and yield (Berahim *et al.*, 2021). The chlorophyll index plays a vital role in assessing plant N status. Over the past two decades, SPAD-measured rice N status has been widely used to ascertain N requirements at different growth stages and optimize grain yield and N use efficiency (Rueda *et al.*, 2016).

Statistical analysis in this study indicates that organically cultivated rice exhibits a significantly higher chlorophyll index than its inorganically cultivated counterpart as the plants mature. The SPAD values were recorded at 41.97 units for organic rice and 40.62 units for inorganic rice, respectively (Figure 1). The organic plant attains a height of 83.23 cm, while the inorganic counterpart reaches 81.31 cm (Figure 2). The number of inorganic tillers is 15.17, whereas the organic variant has 12.72 tillers (Figure 3). SPAD values, plant height, and the number of tillers increased significantly by 3.22%, 2.31%, and 16.12%, respectively, upon entering the vegetative phase. The observed differences in plant height, number of tillers, and SPAD values between the organic and inorganic rice plants can be attributed to the distinct cultivation methods and their impact on plant physiology.

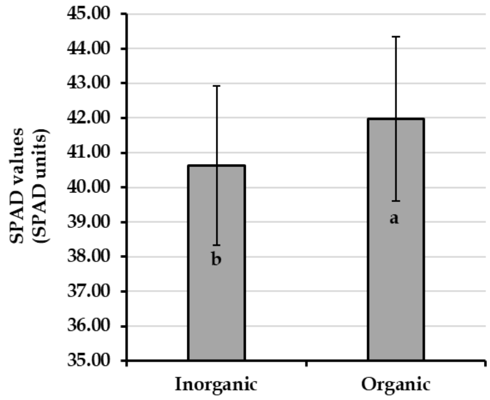

Figure 1. Comparative analysis of rice leaf SPAD values grown under inorganic and organic farming. Different letters within the rectangular bars indicate significant differences based on the LSD test at α 5%; error bars represent the standard deviation.

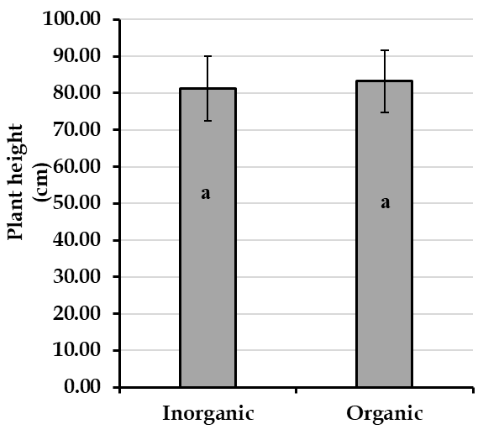

Figure 2. Comparative analysis of rice plant height grown under inorganic and organic farming. Different letters within the rectangular bars indicate significant differences based on the LSD test at α 5%; error bars represent the standard deviation.

Comparative analysis of physiological characteristics of rice. This research underscores the impact of organic and inorganic cultivation methods on water use efficiency within each planting approach. Notably, organic rice plant's WUE exhibited a substantial improvement, registering an increase of 9.12%—from 1591.82 to 1751.50 μmol CO_2 mmol H_2O^{-1} (Figure 4). It is essential to recognize that in a purely hydrological context, WUE has been defined as the ratio of the volume of water used productively (Stanhill, 1986). Agronomic practices associated with organic methods enhance grain yield and contribute to an overall increase in WUE (Mallareddy *et al.*, 2023). Previous studies have emphasized the pivotal role of nitrogen in augmenting agricultural yields, with its utilization and absorption contingent on water availability. Applying organic fertilizer is essential for increasing yield and WUE (Liu *et al.*, 2023).

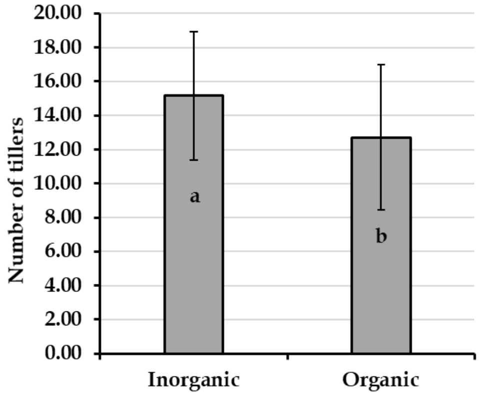

Figure 3. Comparative analysis of a number of tillers in rice plant grown under inorganic and organic farming. Different letters within the rectangular bars indicate significant differences based on the LSD test at α 5%; error bars represent the standard deviation.

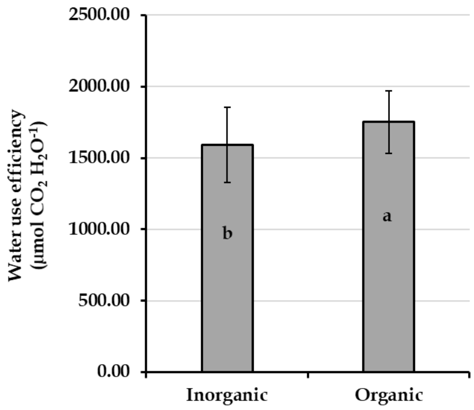

Figure 4. Comparative analysis of the actual water use efficiency of rice plants grown under inorganic and organic farming. Different letters within the rectangular bars indicate significant differences based on the LSD test at α 5%; error bars represent the standard deviation.

A prior investigation identified the critical challenge of managing nutrient availability while improving WUE in rice cultivation under limited water conditions (Ma *et al.*, 2024). Furthermore, it is imperative to anticipate the response of diverse crops' WUE to environmental factors and escalating atmospheric CO_2 levels (Bhattacharya, 2019). Several studies have demonstrated that elevated CO_2 levels not only lead to increased plant biomass but also positively influence water and nitrogen use efficiency (Ullah *et al.*, 2019). In the context of organic rice cultivation, it is noteworthy that irrigation practices contribute to a 56% reduction in runoff, consequently augmenting water storage in the soil compared to inorganic methods (de Avila *et al.*, 2015). In this research, inorganic rice cultivation showed comparable efficiency in using carbon dioxide to the organic method, with both methods obtaining similar values of 0.022 and 0.020 µmol CO_2 mmol H_2O^{-1} (Figure 5). The plant responded to atmospheric CO_2 by increasing its photosynthesis rate, which supported the growth and yield of crops (Priyadarsini *et al.*, 2024).

Neither inorganic nor organic cultivation showed a significant effect on CO_2 efficiency. The level of CO_2 in rice has been simulated in climate change studies using a "current/high CO_2" approach, where crop models are put at current CO_2 (usually 0.05–0,10 µmol CO_2 mmol H_2O^{-1}) and under the high CO_2 concentration scenario (Neeharika *et al.*, 2024). The reduction in nutrient uptake can be attributed to diminished plant transpiration under conditions of CO_2 efficiency, altering the nutrient uptake from the soil, typically translocated via water (Balbinot *et al.*, 2021). Remarkably, plants cultivated in organic conditions acquired higher levels of CO_2 during specific planting phases, thereby potentially mitigating the adverse impact of increased CO_2 concentrations in a single planting cycle on the nutrient content within the plant's growing environment (Lv *et al.*, 2022).

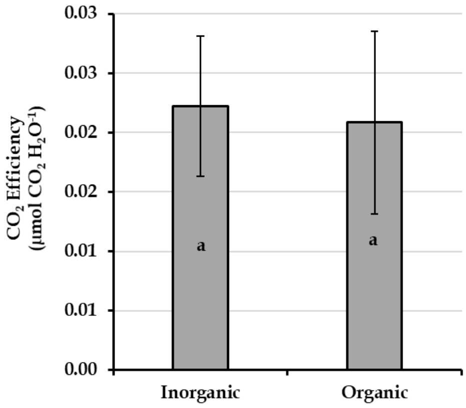

Figure 5. Comparative analysis of the efficiency of actual carbon dioxide in rice plants grown under inorganic and organic farming. Different letters within the rectangular bars indicate significant differences based on the LSD test at α 5%; error bars represent the standard deviation.

Conclusion

This research presents a comparative analysis of morpho-physiological characteristics as affected by organic and inorganic farming methods in 45-day-old greenhouse-grown plants. Rice grown under organic farming has a significantly higher chlorophyll index and actual WUE of rather than those under inorganic farming. While inorganic farming has a higher tiller number than organic ones. Both farming systems have insignificant differences in the variable of CO_2 efficiency and plant height.

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The effect of seed provenance and organic fertilizer types on growth and yield of G_2 potato (*Solanum tuberosum* L. cv. Medians) seeds in Jatinangor medium plains

Abstract. Potato growth is influenced by the seed provenance and fertilization, but the availability of high quality potato seeds is still limited. To overcome those challenges, solutions include using temperature-tolerant potato varieties from medium plains and applying organic fertilizers to improve potato growth and yield. The purpose of this research was to determine the effect of the interaction between seed provenance and application of organic fertilizers on the growth and yield of G_2 potato seeds of the Medians cultivar in the Jatinangor medium plains. The research was conducted from June to October 2023 at the Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor. The research design used was Factorial Randomized Group Design (RBD). The first factor is the provenance of the potato seeds from the medium and high plains. The second factor is the type of organic fertilizer, namely chicken manure, cow manure, and guano fertilizer. The results showed that there was no interaction between the seed provenance and types of organic fertilizer on the growth and yield of G_2 potato seeds of the Medians cultivar in Jatinangor medium plains. The use of seeds from medium plains had a significant effect on plant height, plant dry weight, and the percentage of stolons forming tubers. The application of cow manures with a dose of 225 g gave the best effect on leaf area, the number of tubers, and the weight of tubers per plant.

Keywords: Medium plains · Organic fertilizer · Potato seed · Seed provenance

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Introduction

Potato (*Solanum tuberosum* L.) is one of the vegetable crops commonly consumed and cultivated by the society giving it high commercial value. Potatoes can grow well in high plain areas with an altitude of 1,000-2,000 metres above sea level with humid and cold climatic conditions, soil that is crumbly, loose, and contains organic matter (Hamdani *et al.*, 2020).

Potato seed is one of the most expensive components of potato production, which can cost up to 30-40% of the total cost of potato production (Fuglie *et al.,* 2006; de Putter *et al.,* 2014). Hamdani *et al.* (2020) stated that the availability of quality seed potatoes in Indonesia is still unable to meet the needs of farmers due to the limitations of land use in the high plain areas, one of which is due to land conversion. Efforts can be made to expand the area of potato cultivation to the medium plains, which are widely available in Indonesia.

Originally planted in the high plain areas, potato seeds that are later planted in the medium plains with hotter temperatures will experience obstacles in their growth and development because they require a longer adaptation period because the seed provenance affects its adaptability and can inhibit potato seedling initiation (Mubarok *et al.*, 2022). A new potato cultivar such as the Medians cultivar that has more resilient to high temperatures must be developed so that the potato plants can grow well in the medium plains (Muthoni & Kabira, 2015).

Another factor that must be considered in potato cultivation is fertilization. The application of fertilizers containing essential nutrients, especially macro-nutrients such as high N, P and K can also help increase the yield and growth of potato plants (Muhibuddin *et al*, 2022). Organic fertilizers containing the sufficient macro and micronutrients will be able to increase the vigor, viability, and storage capacity of potato seeds. The application of organic fertilizers will also be able to improve the soil fertility, soil structure, and increasing water holding capacity that allow potatoes to grow optimally (Ahmed *et al.*, 2019). The objective of this research was to determine the interaction between potato seed provenance and different types of organic fertilizers on the growth and yield of G_2 potato seed cultivar Medians in medium plains.

Materials and Methods

The research was conducted from June to October 2023 in a plastic house located at Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency (6˚54′52.9"S, 107˚46′16.6"E) with an altitude of ± 752 metres above sea level (masl). The materials used in this study include G_1 potato seed cultivar Medians originating from the medium plains of Jatinangor and the highlands of Garut Regency, top soil of Inceptisol, polybags measuring 30 cm x 30 cm, chicken manure (already mature, odorless, crumbly structure, light brown to grey color), cow manure (already mature, odorless, dark brown color), guano fertilizer from Gunungkidul Regency (light brown color, odorless), bamboo stakes, plastic ropes, labels, silver black plastic mulch (MPHP), brown envelopes, fumigant Basamid 98 GR (Dazomet 98%), fungicide Antracol 70 WP (Propineb 70%), Urea (46% N), SP-36 (36% P_2O_5), KCl (60% K_2O), and insecticide Curacron (Profenofos 500 g/L). The tools used included hoes, plastic buckets, embraters, scissors, cutters, rulers, hole punches, digital and analytical scales, thermohygrometers, ovens, and some supporting equipment such as stationery and documentation equipment. The research design used was Factorial Randomised Group Design (RBD) consisting of two factors, namely 2 potato seed provenances (A) and 3 types of organic fertilizers (P).

a_1 : G_1 Potato Seeds cultivar Medians from Medium Plains
a_2 : G_1 Potato Seeds cultivar Medians from High Plains
p_1 : Chicken Manure
p_2 : Cow Manure
p_3 : Guano Fertilizer

There were 6 treatment combinations that were repeated 4 times, resulting in 24 experimental units. Each experimental unit consisted of 5 plants, resulting in a total of 120 plants. The planting media used were chicken manure, cow manure, guano fertilizer, and Inceptisol soil that had been sterilized by adding Basamid 98 GR fumigant as much as 500 g for 1 ton of soil and incubated for 1 week.

The preparation of planting media and application of organic fertilizers were carried out by mixing soil with organic fertilizers at a dose of 0.225 kg for each type of organic fertilizer and 2.8 kg of soil in a polybag arranged with a spacing of 30 x 30 cm. Samples were taken by purposive sampling method, where samples were selected by their better attributes such as higher number of leafs and plant height. Each polybag has 3 kg maximum weight consists of a mixture of soil and organic fertilizer. Potato seeds were first soaked in Antracol 70 WP fungicide solution for 10 to 15 minutes. The planting of potato seeds in polybags was done in the morning, starting with watering the planting medium with water until it was moist, then each polybag was planted with 1 potato seed. Fertilizers used in this study include Urea (46% N), SP-36 (36% P_2O_5), and KCl (60% K_2O). The dosage of Urea fertilizer was 300 kg/ha which was divided into two applications of 150 kg/ha each, SP-36 as much as 150 kg/ha, and KCl as much as 100 kg/ha. Urea fertilizer was applied when the plants were aged 7 days after planting (DAP) and 30 DAP at 1.7 g/polybag each, while SP-36 fertilizer at 1.7 g/polybag and KCl at 1.1 g/polybag were applied when the plants were 7 DAP. Plant maintenance consists of watering, weeding, fertilizing, installing stakes, tying plants, pruning flowers, and controlling plant disturbing organisms (PDOs).

The observation parameters consisted of plant height (cm), plant dry weight (g), shoot root ratio (SRR), leaf area (cm^2), leaf area index, number of stolons and percentage of stolons forming tubers (%), number of tubers per plant, tuber weight per plant (g), and percentage of tubers per seed quality grade (%). Plant height was measured by ruler from the base of the stem to the highest growing point. Plant dry weight and shoot root ratio was measured by destructive method, where plant samples were washed, oven-dried, and then weighed. Leaf area was measured by Gravimetric method. Leaf area index was measured by calculating the average leaf area per clump divided by the area of the plant canopy.

The number of stolons was measured by adding up the primary and secondary stolons, while percentage of stolons forming tubers was measured by dividing the total number of tubers formed with the number of stolons and then multiplied by 100%. Both were measured by destructive method. The number of tubers per plant was calculated manually at harvest time. Tuber weight per plant was measured by weighing all of the tubers produced by each plant using a digital scale. Percentage of tubers per seed quality grade was measured by adding up all of the potato seeds from the same class category, divided by the total of seed yield, then multiplied by 100%. According to Direktorat Perbenihan Hortikultura (2014), G_1 potato seeds classification were divided into three class categories, namely Small (< 40 g), Medium (40 – 90 g), and Large (> 90 g – 120 g).

The influence of the treatments were tested with the F test at a significance level of 5%, while to test the difference in mean values between treatments was carried out with the Duncan Multiple Range Test at a significance level of 5%. Data analysis was conducted using SPSS software version 27.

Results and Discussion

Plant Height (cm) and Plant Dry Weight (g). Plant height observations were conducted when the plants were 2, 4, 6, and 8 weeks after planting (WAP), while observations of plant dry weight were conducted when plant destructive on 50 and 60 days after planting (DAP).

The results of the analysis showed that there was no interaction effect between seed provenances and application of organic fertilizer types on plant height and dry weight, but independently the treatment of seed provenances and application of organic fertilizer types had a significant effect on plant height and dry weight (Table 1). Plant height and dry weight is one indicator of plant growth because the better the plant growth, the height and dry weight of the plant will increase.

G_1 potato seeds from the medium plains were able to produce significantly higher plant height and dry weight than those from the high plains. This could allegedly occur because the G_1 potato seeds from the medium plains are the result of previous potato planting carried out in the same environment, so they have better adaptability. Plant height is known to be influenced by several factors, one of which is the adaptability of plants to the environment in which they grow. The adaptability of seeds is thought to have a relationship with gibberellin content, where seeds from the medium plains have a higher gibberellin content. According to Alexopoulos *et al.* (2017), gibberellin may induce faster potato seeds dormancy breakage and affecting the sprout growth. It has a function to

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stimulate potato germination, so the higher the gibberellin content, the faster the potato seeds will sprout.

Based on Table 1, the application of cow manure could produce the highest potato plant height compared to other treatments because cow manure generally contains higher nutrients and cellulose levels compared to other types of organic fertilizers, such as 3.00% N; 2.00% P_2O_5; and 1.00% K_2O (Gilroyed, et al., 2015). Chicken manure may contain N 1.00%, P_2O_5 0.80%, K_2O 0.04% (Dani *et al*., 2021). Pure guano fertilizer may contain higher nutrients, such as N 8.0-13%, P 5-12%, K 1.5-2.5%, Ca 7.5-11%, Mg 0.5-1%, and S 2-3.5% but nowadays it is usually already mixed with other ingredients, such as rice husks, so it could lower the nutrients (Karimou *et al*., 2020). Macro nutrients such as N, P, and K are needed by potato plants to encourage vegetative growth, such as the stem which will make potato plants grow taller. Cow manure also contains organic matter that can improve soil aeration and drainage and high water content so that nutrients can be absorbed by plants better.

Shoot Root Ratio, Leaf Area (cm^2), and Leaf Area Index. The results of the analysis showed that there was no significant effect of the use of seed provenances and the application of organic fertilizer types on the shoot root ratio, but the treatment of seed provenances and application of organic fertilizer types had a significant effect independently on the parameters of leaf area and leaf area index (Table 2).

Shoot root ratio is the ratio between the dry weight of the upper part of the plant (shoot) which functions as a place for photosynthesis and the dry weight of the roots which functions as a place for nutrient absorption. The treatment of seed provenances and organic fertilizer application did not show significant differences on shoot root ratio. This might occur because the macro nutrient content contained in organic fertilizers, such as N and P elements will increase the supply of nutrients to the shoot part of the plant which is higher due to the influence of high temperatures compared to the growth of the root part, thus increasing the value of the shoot root ratio. All shoot root ratio values produced are more than 1, meaning that plant growth tends towards shoot or upper part or the plant.

Leaf area is closely related to leaf area index, where the wider the plant leaves, the higher the leaf area index value. Based on the data in Table 2, it is known that the treatment of potato seed provenances from the high plains produced lower leaf area and leaf area index compared to those from the medium plains. The low value of leaf area and leaf area index is thought to be due to the response of plants to deal with environmental conditions that have high temperatures by reducing the transpiration rate in the leaves, resulting in small leaves. In agreement with Parker (2020), leaf area and leaf area index are influenced by weather, such as temperature.

The application of cow manure was able to produce the highest value of leaf area and leaf area index compared to other treatments because the essential macro and micronutrients contained in cow manure are higher than other types of organic fertilizers, so as to accelerate plant leaf growth due to an increase in the photosynthesis process. The Nitrogen plays a role in increasing chlorophyll production in leaves, thereby increasing the surface area of the leaves, while Phosphorus plays a role in increasing the number of leaves and increasing leaf area of potato (Sun *et al*., 2015). This is in line with the results of research conducted by Arzad *et al*. (2017) which showed that the application of cow manure can increase the number of leaves that can increase the total leaf area of plants because the availability of nutrients and the cation exchange capacity of the soil also increases so that the absorption of nutrients by plants is more optimal.

G_1 potato seeds from the high plains were able to produce a greater number of stolons, but produced a lower percentage of stolons forming tubers compared to seeds from the medium plains. High environmental temperatures can cause higher gibberellin synthesis on potato that can promote the vegetative growth, but reducing the tubers growth (Caliskan *et al*., 2021). Gibberellin, which acts as a trigger for cell division and elongation, will trigger the stolons to continue growing rather than the enlargement of the stolons into potato tubers, so that the formation of potato tubers will be inhibited.

Table 1. Effects of seed provenances and organic fertilizer types on plant height (cm) and plant dry weight (g).

Treatments	Plant Height (cm)				Plant Dry Weight (g)	
	2 WAP	4 WAP	6 WAP	8 WAP	50 DAP	60 DAP
G_1 Potato Seed Provenances (A)						
a_1 : Seed from Medium Plains	8.88 b	16.90 b	23.47 b	32.19 b	5.01 b	10.00 b
a_2 : Seed from High Plains	4.25 a	14.20 a	20.51 a	25.78 a	4.02 a	9.11 a
Types of Organic Fertilizers (P)						
p_1 : Chicken Manure	4.83 a	13.37 a	20.04 a	27.49 a	3.41 a	8.59 a
p_2 : Cow Manure	8.51 b	18.20 b	24.44 b	31.73 b	5.68 c	10.90 b
p_3 : Guano Fertilizer	6.35 a	15.09 a	21.49 a	27.73 a	4.46 b	9.17 a

Description : Mean values followed by notations with the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% significance level.

Table 2. Effects of seed provenances and organic fertilizer types on shoot root ratio, leaf area (cm^2), and leaf area index.

Treatments	Shoot Root Ratio		Leaf Area (cm^2)	Leaf Area Index
	50 DAP	60 DAP		
G_1 Potato Seed Provenances (A)				
a_1 : Seed from Medium Plains	3.77 a	4.53 a	586.28 b	0.65 b
a_2 : Seed from High Plains	3.56 a	4.05 a	293.01 a	0.33 a
Types of Organic Fertilizers (P)				
p_1 : Chicken Manure	3.20 a	3.98 a	231.57 a	0.26 a
p_2 : Cow Manure	4.56 a	4.72 a	690.25 b	0.77 b
p_3 : Guano Fertilizer	3.25 a	4.17 a	397.11 a	0.44 a

Description : Mean values followed by notations with the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% significance level.

The data in Table 3 also showed that the application of organic fertilizer did not significantly different on the number of stolons and the percentage of stolons forming tubers. The application of organic fertilizers could improve soil structure, soil permeability, soil porosity, soil aggregation, water holding capacity, and regulates soil pH which can make the soil in an optimal condition for the growth and development of potato tubers that may stimulate the growth of stolons in large quantities (Bhujel *et al.*, 2021).

Number of Tubers and Tubers Weight per Plant. The analysis showed that there was no interaction effect between the use of potato seed provenances and the application of organic fertilizer types on the weight of potato tubers and the number of potato tubers per plant, but independently the treatment of potato seed provenances and the application of organic fertilizer types had a significant effect on the number of potato tubers and the weight of potato tubers per plant (Table 4).

G_1 potato seeds from the medium plains were able to produce a higher number of potato tubers and the weight of potato tubers per plant compared to those from the high plains because they have better adaptation to high temperature environments, so that the formation and development of potato tubers become more optimal. This is in accordance with the opinion expressed by Bischoff *et al.* (2008) which shows that seeds planted in their area of origin will provide better crop yields compared to seeds that are not from the same area due to genetic differences influenced by specific environmental conditions.

The application of cow manure was able to produce the highest weight and number of potato tubers per plant due to the high content of macro nutrients, such as N, P, and K.

The element of Nitrogen plays a role potato tubers growth and yield, Phosphorus plays a role in root growth and development, and Potassium for the potato tubers production and quality (Parganiha *et al.,* 2022). This is in line with the results of research conducted by Sufianto (2013) which showed that the application of cow manure at a dose of 20 tons/ha was able to produce the highest number

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of potato tubers per plant and the weight of potato tubers per plant compared to the application of chicken and goat manure, which were 10.96 knols and 400.04 g, respectively. According to Hamdani *et al*. (2020), the number of potato tubers is influenced by other factors such as the number of stolons formed, while the weight of potato tubers is influenced by the amount of photosynthetic products stored in the tubers in the form of food reserves.

Percentage of Tubers per Seed Quality Grade. The results of the analysis showed that there was no interaction effect between the use of potato seed provenances and the application of types of organic fertilizers on the percentage of potato tubers per seed quality grade (Table 5). Based on the results of the analysis, it is known that the treatment of potato seed provenances and the application of organic fertilizer types did not have a significant effect on the percentage of potato tubers per seed quality grade. The data in Table 5 shows that all potato tubers produced were S-sized or weighed < 40 g. This is thought to occur because at high temperatures, the flow of carbohydrates to the potato tubers will decrease because the energy released will be used for the formation of the upper part of the plant, resulting in small-sized potato tubers. This is in line with the results of research conducted by Muhibuddin *et al*. (2022) which showed that high temperatures can affect the yield of potato.

The competition between a large number of sweet potatoes in obtaining photosynthetic products is also thought to cause the development of potato tubers to be not optimal. Muhibuddin *et al.* (2022) stated that if the number of tubers formed is large, there will be competition between tubers in obtaining assimilates, so that many prospective tubers cannot develop because they experience a lack of assimilates which then produce small-sized tubers.

The G1 potato seeds from medium plains can be used in further research to be planted in highlands with the aim of studying in more depth the effect of seed adaptability on potato growth and yield.

Table 3. Effects of seed provenances and organic fertilizer types on number of stolons and Percentage of Stolons Forming Tubers (%).

Treatments	Number of Stolons		Percentage of Stolons Forming Tubers (%)	
	50 DAP	60 DAP	50 DAP	60 DAP
G_1 Potato Seed Provenances (A)				
a_1 : Seed from Medium Plains	10.92 a	11.92 a	37.35 b	43.05 b
a_2 : Seed from High Plains	12.75 b	13.50 b	26.43 a	35.64 a
Types of Organic Fertilizers (P)				
p_1 : Chicken Manure	11.38 a	12.38 a	28.85 a	37.51 a
p_2 : Cow Manure	12.38 a	13.13 a	36.41 a	41.86 a
p_3 : Guano Fertilizer	11.75 a	12.63 a	30.40 a	38.67 a

Description : Mean values followed by notations with the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% significance level.

Table 4. Effects of seed provenances and organic fertilizer types on number of tubers and tubers weight per plant.

Treatments	Number of Tubers	Tubers Weight per Plant (g)
G1 Potato Seed Provenances (A)		
a_1 : Seed from Medium Plains	4.78 b	69.53 b
a_2 : Seed from High Plains	3.87 a	44.19 a
Types of Organic Fertilizers (P)		
p_1 : Chicken Manure	3.12 a	51.09 a
p_2 : Cow Manure	5.79 c	62.58 c
p_3 : Guano Fertilizer	4.06 b	56.91 b

Description : Mean values followed by notations with the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% significance level.

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Table 5. Effects of seed provenances and organic fertilizer types on percentage of tubers per seed quality grade.

Treatments			Percentage of Tubers per Seed Quality Grade		
			S (%)	M (%)	L (%)
G1 Potato Seed Provenances (A)					
a_1	:	Seed from Medium Plains	100.00 a	0.00 a	0.00 a
a_2	:	Seed from High Plains	100.00 a	0.00 a	0.00 a
Types of Organic Fertilizers (P)					
p_1	:	Chicken Manure	100.00 a	0.00 a	0.00 a
p_2	:	Cow Manure	100.00 a	0.00 a	0.00 a
p_3	:	Guano Fertilizer	100.00 a	0.00 a	0.00 a

Description : Mean values followed by notations with the same letter are not significantly different based on Duncan's Multiple Range Test at the 5% significance level.

Conclusions

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

1. There was a non-significant interaction between the application of organic fertilizer types and seed provenance that could increase the growth and yield of G2 potato seed cultivar Medians in the Jatinangor medium plain.
2. The use of G1 potato seeds from the medium plains gave a better effect than potato seeds from the high plains on the parameters of plant height, dry weight, leaf area, leaf area index, percentage of stolons forming tubers, number of tubers per plant, and.
3. The application of cow manure gave the highest average value on plant height, dry weight, leaf area, leaf area index, number of tubers per plant, and tubers weight per plant.

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Current status and the significance of local wisdom biofertilizer in enhancing soil health and crop productivity for sustainable agriculture: A systematic literature review

Abstract. Soil fertility is recognized as a crucial factor in supporting plant growth and productivity. The utilization of biofertilizers as environmentally friendly fertilizers is aimed at enhancing soil fertility and plant productivity. This study aims to explore the potential of local material for developing local wisdom biofertilizers (LWB) for achieving sustainable agriculture. A systematic literature review was conducted using bibliometric analysis, Preferred Reporting Items for Systematic Reviews, and Meta-Analyses (PRISMA) method, employing the Scopus search engine with the keywords "local AND wisdom AND biofertilizer" OR "biofertilizer" OR "local AND microorganism" OR "soil AND health OR crop AND productivity OR sustainable agriculture". The search yielded 704 articles, of which 11 were deemed eligible after selection. Based on the literature review, it was found that there are local materials, including fish waste, seaweed, Azolla, fruit waste, *Moringa oleifera*, microalga, bamboo roots, banana hump, golden snail, mangrove leaves, fruit, and vegetable waste that can be used as raw materials for LWB to improve soil health, plant growth, and productivity. The development of LWB as a new fertilizer technology faces challenges such as lack of regulations, low public trust, limited farmer awareness, weak promotion, and raw material shortages. Further research is needed to intensively study and enhance the effectiveness of LWB through enrichment using beneficial microorganisms.

Keywords: Indonesia · Local wisdom biofertilizer · PRISMA · Sustainable agriculture · Waste management

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Introduction

The demand for food, both in Indonesia and globally, is expected to increase in line with population growth. By mid-2023, the population of Indonesia had reached 278 million people (BPS, 2023). The success of agricultural production is vital in meeting food demands. Plant productivity is influenced by both internal and external factors. Internal factors encompass plant genetics (Soeparjono, 2016), while external factors include soil conditions and climate variables such as humidity, rainfall, and environmental temperature (Triharyanto et al., 2020). The increase in average temperatures and occurrences of extreme global climatic phenomena directly affect crop yield stability and production (Setyaningrum et al., 2024). Various efforts have been made to enhance crop production, including agricultural intensification and extensification (Subaedah & Aladin, 2016).

The increase in production through extensification (expansion of land) faces challenges due to high competition for land use, often leading to the utilization of marginal lands. Utilizing marginal lands for agricultural activities poses various constraints, including low soil fertility (Subaedah & Aladin, 2016). The low availability of nutrients during the production phase impedes several plant metabolic processes, thus hindering flower formation, reducing seed quantity, and lowering crop yields (Wei et al., 2017). Improving nutrient availability in the soil can be achieved through fertilization. Fertilizer application aims to supplement the required nutrients for plants, as the nutrients present in the soil are insufficient to support optimal plant growth (Hamid & Tanweer, 2021).

Fertilizers are substances that provide essential nutrients for plant growth (Simanjuntak & Setiawan, 2021). In recent years, the use of agricultural chemicals such as inorganic fertilizers and pesticides has significantly increased (Muktamar et al., 2016). Excessive use of inorganic fertilizers leads to soil fertility decline (Dorota et al., 2020). Long-term application of high doses of inorganic fertilizers can increase soil compaction, reduce organic matter, and disrupt nutrient balance, thus decreasing soil fertility. Moreover, inorganic fertilizers are expensive and have limited availability (Yang et al., 2022). A potential technology that can substitute or even replace the use of inorganic fertilizers is local wisdom biofertilizer.

Local wisdom biofertilizer (LWB), also known as local microorganisms, is a fermentation liquid derived from natural materials containing numerous microorganisms capable of transforming organic matter, thus serving as biofertilizers, decomposers, and organic pesticides (Roeswitawati & Ningsih, 2018). LWB, as a biofertilizer, can be used to nourish plant leaves and stimulate plant growth (Retnowati & Katili, 2021). Implementing LWB at the community level can realize the concept of bio-cycling farming. This is because LWB formulations can utilize materials sourced from local wisdom waste, thus recycling environmental waste and benefiting plant growth (Kumar & Gopal, 2015). Materials that can be utilized include cow urine, vegetable waste, fruit waste, banana humps, leaves, and banana stems (Retnowati & Katili, 2021). Additionally, materials such as seaweed waste, molasses, and earthworm castings can also be utilized (Arfarita et al., 2022).

The utilization of locally sourced LWB supports the achievement of sustainable agriculture as it maximizes environmental resources without causing harm. LWB is more resistant to nutrient-leaching processes and can rapidly provide nutrients (Hersanti & Nurusilawati, 2012). LWB not only contains a single type of microorganism but a variety of microorganisms such as *Azospirillum* sp., *Azotobacter* sp., *Rhizobium* sp., *Bacillus* sp., *Pseudomonas* sp., and phosphate-solubilizing bacteria (Roeswitawati & Ningsih, 2018). Implementing this technique is more environmentally friendly and ensures safe and healthy agricultural products. This systematic literature review aims to explore the current status and importance of LWB in sustainable agriculture practice. By systematically analyzing relevant literature, this research aims to assess the effectiveness of LWB in enhancing soil fertility, promoting plant growth, and encouraging sustainable agricultural practices. Through a comprehensive review of existing research, this research provides insights into the potential, benefits, and challenges associated with the adoption of LWB in sustainable agriculture.

Materials and Methods

Search Analysis in Scopus. Searches were conducted in the Scopus database for bibliometric analysis and systematic literature review. The search results were retrieved from the Scopus database using a combination of keywords such as "local AND wisdom AND biofertilizer" OR "biofertilizer" OR "local AND microorganism" OR "soil AND health OR crop AND productivity OR sustainable agriculture". The data on the number of articles published by different categories, such as journals, year, and language, were exported and analyzed.

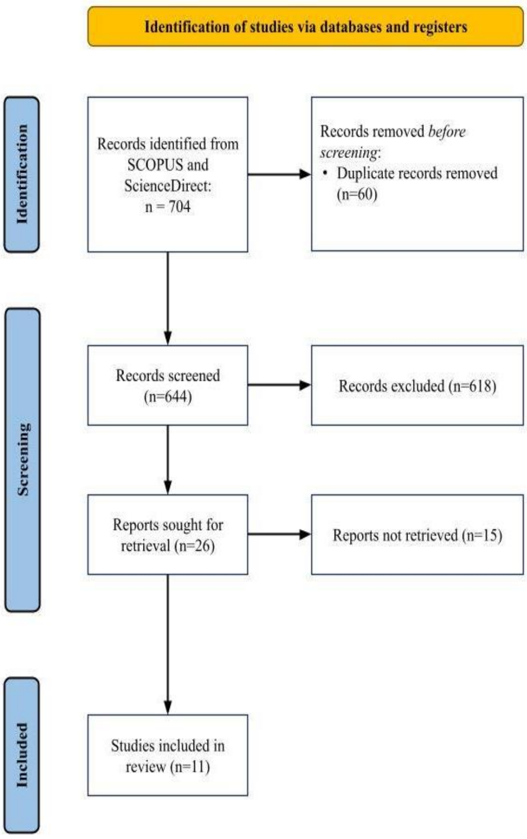

Figure 1. PRISMA flowchart reporting the article selection process for the systematic literature review

Systematic Literature Review using PRISMA. The articles were retrieved from the SCOPUS database and were restricted to the last five years (2020–2024). The relevant articles from the database into ris format for further analysis. Articles published in English alone were included in the search. Inclusion and exclusion criteria are tabulated in Table 1. Data retrieved from Scopus were then assessed using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow guidelines or Systematic Literature Review (Figure 1). Duplications were eliminated using Mendeley Reference Manager 2.107.0. Eligibility of the documents included was based on the abstract related to the topic of the article; documents not related to the topic were categorized as ineligible and thus not included for further content analysis (Ariani & Simarmata, 2023)

Results and Discussion

Bibliometric Analysis by VOSViewer. Bibliometric data analysis employed VOSviewer to uncover emerging study themes and research trends from the last five years. By visually mapping keyword co-occurrences through binary counting, 46 key keywords were identified, setting a minimum threshold of 25 co-occurrences among document keywords in research articles. These significant terms effectively reflect the research directions pursued by scientists investigating local wisdom biofertilizers for sustainable agriculture.

Based on Figure 2, the researcher's focus is divided into three parts, firstly those who use LWB as fertilizer for crop yield (cluster 1), secondly those who study LWB and their relationship with rhizobacteria (cluster 2), and lastly those who investigate plant physiology particularly plant's response to salt stress with LWB application (cluster 3). Cluster 1, indicated in red color, consists of terms such as arbuscular mycorrhizal fungi, cultivation, fertilization, fertilizer, fruit, grain yield, Indonesia, inorganic fertilizer, microorganism, nutrient, organic fertilizer, organic matter, potassium, productivity, rhizobium, soil fertility, sustainable agriculture, and yield. Cluster 2, indicated in green color, consists of terms such as acetic acid, bacillus, IAA, PGPR, phosphate, phosphate solubilization, plant growth, pseudomonas, rhizobacteria, rhizobacterium, rhizosphere, rice, root, seedling, shoot, siderophore, tomato. Cluster 3, indicated in blue color, consists of terms such as chlorophyll, chlorophyll content, foliar application, fresh weight, leaf area, plant height, pod, root length, salinity, salt stress, weight. When examining the publication timeline in Figure 3, it becomes evident that research on local wisdom biofertilizers (LWB) focusing on microorganisms, organic fertilizers, and their impact on plant responses to salt stress emerged as prominent topics towards late 2022 (indicated by yellow color). Notably, publications from 2023 to 2024 did not feature prominently in Figure 3, indicating their minority representation. This suggests a lower number of publications during 2023-2024 compared to the earlier period of 2020-2022..

Table 1. Inclusion and exclusion criteria used in this study

Criteria	Inclusion	Exclusion
Relevance topics	Journal with focus on local wisdom biofertilizer	Journal without core focus on local wisdom biofertilizer
Date of publication	2020-2024	Years before 2020
Type of Publication	Research article	Books chapter, Encyclopedia, News, Conference abstracts
Language of publication	English	All other language
Access	Open access	No open access
Databases	Scopus and ScienceDirect	Article that are not indexed by Scopus

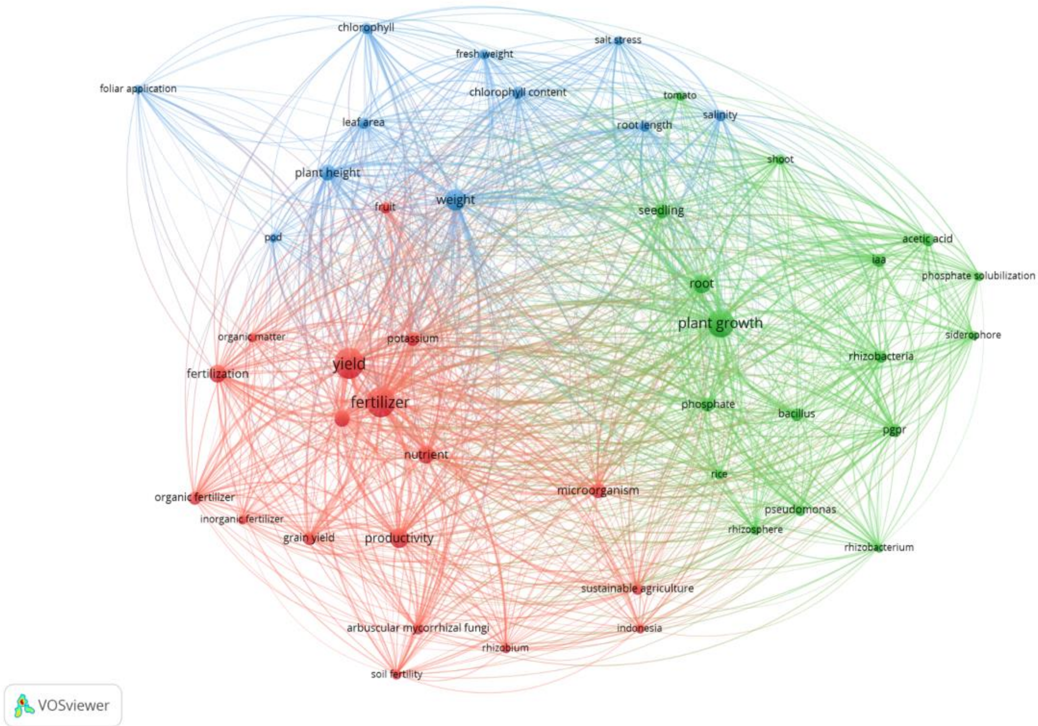

Figure 2. A network visualization of the co-occurrence mapping of pertinent keywords related to research on local wisdom biofertilizer

These results showed that LWB contributes to enhancing soil health and crop productivity party as well as answering the problem of abiotic stress factors in plants. Exploring the potential of blending traditional agricultural techniques with modern farming practices to pave the way for sustainable agriculture (Sekhar et al., 2024). For instance, blue-green algae can contribute 30–40 kg N/ ha, whereas the use of phosphate-solubilizing microbes can potentially cut down chemical phosphorus fertilizer usage by half which is potential to boost soil health (Susanti et al., 2024). Significantly, the combination of *Alcaligenes faecalis* and *Metabacillus indicus* with half of the recommended doses of N, P, and K fertilizers resulted in a substantial increase in rice grain yields (Fatema et al., 2024). *Pseudomonas* spp., isolated from cotton rhizospheric soil, exhibit potential for suppressing plant diseases and could be beneficially used in seed biopriming to enhance the growth and productivity of *Triticum aestivum* L. plants, particularly in alkaline soils (Kankariya et al., 2024).

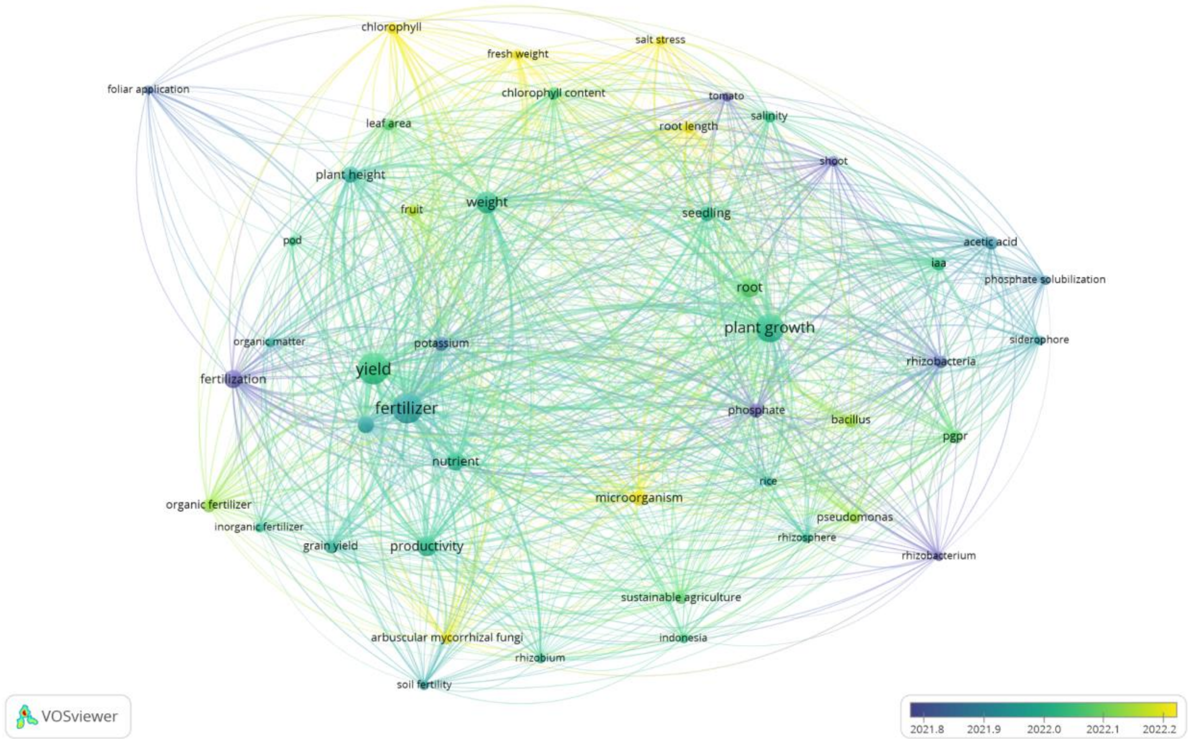

Figure 3. An overlay visualization of the publication year mapping related to research on local wisdom biofertilizer

Inorganic Fertilizer VS Local Wisdom Biofertilizer. The use of inorganic fertilizer (IF) aims to increase crop productivity, yet excessive utilization can have negative impacts on soil and the environment (Rossel & Bouma, 2016). Intensive and prolonged IF application can decrease soil organic matter, disrupt soil structure, and lead to environmental pollution. Reduction in organic matter results in decreased availability of N, P, and K, leading to toxin accumulation for plants and reduced nutrient availability in the soil (Soebandiono et al., 2021). Moreover, it can diminish soil microorganisms and increase soil degradation (Savci, 2012). One alternative to mitigate IF usage is substituting it with local wisdom biofertilizer.

LWB, also known as local microorganisms, is a fermentation solution derived from various sources, including plants and animals, containing carbohydrates (sugar), proteins, minerals, and vitamins. Utilizable materials include rice, fruits, eggs, milk, snails, fish waste, shrimp waste, among others (Mursalim et al., 2018). Bacteria like snails and fruits contain carbohydrates such as rice washing water, coconut water, and glucose sources like molasses or sugar water (Dewilda et al., 2021). LWB, as a liquid organic fertilizer, is beneficial for the environment as it mitigates environmental pollution and provides soil microorganisms useful for decomposing organic matter into plant-absorbable fertilizers. LWB contains macro and micronutrients and bacteria capable of stimulating plant growth, decomposing organic matter, and controlling plant diseases (Tulung et al., 2023).

LWB can substitute the role of IF and other commercial fertilizers, its production is not difficult and is more cost-effective since it utilizes readily available materials (Soebandiono et al., 2021). LWB can serve as a raw material for fertilizers. Microbial technology utilization is not only essential for plants but also beneficial for decomposing organic materials, unused agricultural materials, household waste disposal, and industrial waste (Manullang et al., 2018). Thus, LWB utilization can reduce heavy reliance on IF, enhance profit income by reducing production costs, and improve yields (Soebandiono et al., 2021).

Systematic Literature Review. A literature search conducted on the Scopus database using "local AND wisdom AND biofertilizer" OR

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"biofertilizer" OR "local AND microorganism" OR "soil AND health OR crop AND productivity OR sustainable agriculture" resulted in 704 research articles. After data cleaning of duplicates and ineligible entries, 644 articles remained. When exclusion and inclusion criteria were applied to relevant articles based on title and abstract screening, 26 were excluded. The final list of 11 research articles analyzed systematically is presented in Table 2.

Seaweed Waste. Seaweed waste is generated from seaweed processing plants. This material can be utilized as organic fertilizer and soil conditioner. The application of seaweed extract on plants can affect germination, increase resistance to stresses (biotic and abiotic stresses), and enhance yields (Prithiviraj, 2009). Seaweed extract consists of water (27.8%), ash (22.25%), protein (5.4%), fat (8.6%), carbohydrates (33.3%), and crude fiber (3%). Additionally, seaweed extract contains various enzymes, amino acids, nucleic acids, vitamins (A, B, C, D, E, and K), macro minerals (oxygen, nitrogen, selenium, and calcium), and micro minerals (iron, sodium, and magnesium) (Arfarita et al., 2022).

Bamboo Roots. Bamboo roots have the potential as an alternative material for use in LWB production because they contain numerous beneficial bacteria such as *Lactobacillus, Streptococcus, Azotobacter,* and *Azospirillum* (Walida et al., 2019). These bacteria produce secondary metabolites that can support plant growth in the form of phytohormones. The secreted phytohormones can stimulate enzymatic processes, biologically enhancing seed germination and accelerating the synthesis of organic nitrogen compounds in roots (Sodiq et al., 2021).

Table 2. Local potential materials as LWB formula.

Local Material	Crop	Result	Referensi
Fish waste	Cowpea	- Increased the height, root length and yield of cowpea - increased the growth and yield of aerial biomass increased the microbial fertility	Maquén-Perleche et al., 2023
Seaweed	Wheat, lettuce, date palm	- Improves growth parameters and grain yield in wheat - Improve the qualitative and quantitative traits of the lettuce crop - enhancement in growth and phytochemicals of plants Improve growth and development of date palm	Vafa et al., 2021; Rasouli et al., 2022; Parab & Shankhadarwar, 2022;
Azolla	Soybean, rice	- Increases soybean plant growth - Increase rice grain yield, nitrogen uptake and agronomic efficiency of N	Pauline et al., 2022; Marzouk et al., 2024
Fruit waste	*Capsicum frutescens*	- Increase growth and yield of *Capsicum frutescens*	Akpan et al., 2020
Moringa oleifera	Mustard spinach	- Promoted the growth and yield of mustard spinach	Chanthanousone et al., 2022
Microalga	Onion	- Promoted plant growth and increases in bulb caliber and yield of both onion cultivars.	Cordeiro et al., 2022
Indigenous microorganisms, mangoo, and longan	Okra	- Enhance the yeast community in IMO inoculants, indirectly improving okra growth and benefiting the agriculture field in the future	Selvarajoo, 2023
Soaked coconut fiber with cow urine	Rice	- increase fill grain of rice	Yassi et al., 2023

Phytohormones play a crucial role in plant growth and developmental regulation. They are vital regulators of plant growth that control various processes such as cell division, cell differentiation, organogenesis, and morphogenesis (Bielach et al., 2012). Walida et al. (2019) found that applying LWB from bamboo shoots to Jenggo F1 red chili plants affected plant height, stem diameter, leaf number, flowering age, and initial production weight.

Banana Hump. Banana hump is one of the materials used as the main ingredient in LWB due to its content of phytohormones, such as gibberellins and cytokinins, which are beneficial for plant growth (Astuti, 2014). Banana hump contains carbohydrates (66%), starch (45.4%), protein (4.35%), water, and essential minerals (Sitinjak et al., 2018). LWB based on banana hump contains numerous microbes involved in the decomposition of organic materials (Sri et al., 2022). Microbes present in banana hump-based LWB include *Bacillus, Azospirillum, Aspergillus, Azotobacter, Aeromonas*, cellulolytic microbes, and phosphate-solubilizing microbes (Sitinjak et al., 2018). The high level of phenolic acids in banana hump organic liquid provides several elements, such as Al, Ca, and Fe, which are beneficial in the flowering process and seed formation (Soebandiono et al., 2021).

Golden Snail. Golden snail serves as a local resource that can be utilized as an ingredient in LWB. Utilizing golden snails can be a sustainable agricultural management effort as their presence in rice fields represents a pest. LWB from golden snails contains various beneficial microorganisms that support plant growth and development, including *Aspergillus niger, Azotobacter, Azospirillium, Pseudomonas*, and *Staphylococcus* sp. Additionally, it contains phytohormones and essential macro and micronutrients (N, P, K, Ca, Mg, Cu, Zn, Mn, and Fe). Spraying golden snail-based LWB every 1-2 weeks can enhance plant tolerance to drought and increase the average fruit weight per plant by 32% compared to the average fruit weight per tomato plant under optimum conditions (Eliyanti et al., 2022).

Mangrove Leaves. The use of LWB in agriculture can improve plant growth, soil fertility, and pathogen control (Kumar & Gopal, 2015). Fermented LWB can inhibit pathogenic bacteria, antimycotoxins, and antifungals. Antimicrobial substances (such as lactic acid) produced by bacteria are used to inhibit the growth of pathogenic microorganisms (Wang et al., 2015). Mangrove leaf-based LWB contains eight *Bacillus* species (Rahman & Ekasari, 2020). These bacteria can produce several bioactive compounds with broad-spectrum inhibitory activity against pathogens (Sandi & Salasia, 2016). LWB can be used as probiotic and biocontrol agents to suppress the growth of pathogenic bacteria through various mechanisms, one of which is the production of metabolites (Widanarni et al., 2015). Mangrove leaf-based LWB contains alkaloids, saponins, tannins, phenolics, flavonoids, and glycosides (Rahman & Ekasari, 2020). Additionally, it contains flavonoids, phenolics, and tannins that play a crucial role as antioxidant sources (Hamli et al., 2017). Glycosides present in LWB are involved in catalytic or anabolic destruction of microbial cell walls (Rijai, 2016).

Fruit and Vegetable Waste. Waste fruits processed into LWB can stimulate generative plant growth and contain several microorganisms acting as probiotic components (Roeswitawati & Ningsih, 2018). Microorganisms in the fermentation process of vegetable waste LWB include genera such as *Obesumbacterium, Megasphaera, Synthropococcus*, and one identified fungus, *Aspergillus* sp. Cellulolytic bacteria in LWB produce enzymes that can hydrolyze cellulose and cellobiose bonds, including species such as *Syntrophococcus, Sucromutans*, and *Ruminicoccus* (Widyastuti et al., 2021).

The quality of vegetable waste-based LWB, as assessed by physical parameters such as odor and color, shows favorable results. Chemical parameters of vegetable waste LWB tested include pH (3.75), organic matter (2.29%), C (1.33%), total N (0.08%), total P_2O_5 (0.04%), total K_2O (0.10%), and C/N ratio (16.63%) (Indrajaya & Suhartini, 2018). Application of vegetable waste LWB affects stem diameter and plant height. Fermented fruit and vegetable waste-based LWB result in increased guava plant height (Widyastuti et al., 2021). Application of fruit waste LWB on broccoli plants affects flower diameter, wet flower weight, and wet broccoli plant weight, as fruit waste LWB contains higher total nitrogen (Roeswitawati & Ningsih, 2018). Research by Yuliana (2021) shows that LWB based on white cabbage and cabbage waste with a 15% LWB concentration significantly affects soil pH, dry weight, and dry weight of Ipomea reptans Poir shoots.

The addition of other substances such as urine, rice water, fish and shrimp waste, and

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molasses in LWB formulations can enhance the effectiveness of LWB in supporting plant growth, development, and soil health. Urine serves as a source of N, K, and plant growth hormones. Urine can boost plant productivity due to its content of plant growth hormones such as auxin-a, auxin-b, and Indole Acetic Acid (IAA). The presence of auxins can stimulate root formation and promote cell division in the cambial vessels, supporting stem growth (Mudhita et al., 2016). Rice water, or "leri," contains numerous vitamins and minerals commonly utilized by plants. Molasses is a sugar mill waste that can be utilized by microbes as a carbohydrate source (Roeswitawati & Ningsih, 2018). Tuna and shrimp waste have high potassium (K) content, thus their utilization can enhance K levels. Potassium plays a crucial role in photosynthesis and aids in the formation of proteins and cellulose in plant stems (Dewilda et al., 2021).

Fermentation Process of LWB. The fermentation process of LWB can be influenced by several factors such as the type of organic material, pH, temperature, types of microbes, and specific substances that enhance microbial activity, thus facilitating a rapid fermentation process (Roeswitawati & Ningsih, 2018). During the fermentation of LWB, several processes occur, including a decrease in pH. The decrease in pH during fermentation is caused by the activity of microorganisms present in the fermentation solution (Yuliana et al., 2019). During the fermentation process, the pH decreases by 3.4-5.2. The decomposition of organic matter leads to an increase in acidity levels and the production of CO_2 gas. This CO_2 gas forms carbonic acid (H_2CO_3), which readily decomposes into H^+ and H_2CO_3 ions. The H^+ ions affect acidity, resulting in a decrease in pH (an increase in acidity) in the LWB solution (Hunaefi et al., 2013). Furthermore, increased acidity can occur due to the nitrification process, which releases ammonium and hydrogen ions (Ma et al., 2022). Subsequently, microorganisms decompose nitrogen into ammonia (NH_2 or NH_2^+). The ammonification process leads to an increase in pH (Grzyb et al., 2021). At the end of the fermentation process, the solution will reach a neutral pH range, indicating a decrease in nitrogen decomposition due to microbial activity (Ramadhani et al., 2022). Addition of stabilizing agents such as PEG (Polyethylene Glycol) and glycerol can be performed to stabilize the microorganisms present in the solution (Simonin et al., 2015). PEG and glycerol, as additive substrates for biofertilizer formulations, are still limited, especially when combined with extracted solid waste materials. Additionally, glycerol can also prolong the shelf life of biofertilizers (Arfarita et al., 2022).

Local Wisdom Biofertilizer as a Concept of Bio-cycling Farming. Indonesia faces challenges in achieving the Sustainable Development Goals (SDGs) for 2030, particularly in waste management and food security. An innovation addressing waste management while enhancing food security simultaneously is the application of the concept of biocycling farming. Biocycling farming is an agricultural method that integrates and utilizes waste to support farming activities. The development of zero-waste farming concepts aims to optimize household and agricultural waste. Implementation involves leveraging the presence of both plant and animal waste to produce LWB. The environmental impact of this program is derived from the total volume of liquid organic waste processed into LWB, resulting in a reduction in organic waste. A decrease of 750 kg of organic waste has the potential to reduce methane gas (CH_4) emissions by 0.0004 Gg/year, equivalent to 7.88 tons of CO_2 equivalent per year (Firmansyah et al., 2023). Applying fish waste-based LWB to cowpea plants can increase height, root length, and yield by 1, 1.25, and 1.5%, respectively (Maquén-Perleche et al., 2023). Apart from that, the research results of Akpan et al. (2020) show that applying fruit waste-based LWB to hot peppers can increase the field of fresh fruit by 266.84% in the rainy season and 150.84% in the dry season.

Urgency and Challenges of Implementing LWB in Indonesia. Utilizing local resources as raw materials for LWB can foster the spirit of farmer self-sufficiency by enabling them to produce organic fertilizers independently. The use of LWB is a component of organic farming that can reduce the reliance on chemical fertilizers (Jasim et al., 2016). The development of organic agriculture has several benefits such as reducing external input costs, increasing farmer income, enhancing food security, and being more environmentally friendly (Jouzi et al., 2017). Developing LWB is a strategy to realize sustainable agricultural systems in Indonesia through a local wisdom approach. This can be achieved through various efforts such as intensive utilization of local resources (energy and nutrients), enhancing farmer skills, and adopting local and environmentally friendly approaches (Srivastava et al., 2016).

Continued innovation is necessary for the development of LWB with a sustainable and environmentally friendly agricultural approach. These innovations are not only related to the utilization of local resources but also to micro and macro fauna that support plant growth. Addition of beneficial microbes can also be carried out to enhance the effectiveness of LWB in supporting plant growth (Soebandiono et al., 2021). Several challenges in the implementation and development of new fertilizer technologies include lack of regulation, lack of public trust, lack of awareness among farmers, inadequate promotion, and shortage of raw material availability (Naveed et al., 2015). The availability of LWB raw materials must be ensured and supported by the entire community and government. Additionally, the promotion of LWB technology that can influence plant growth and increase productivity is essential (Soebandiono et al., 2021).

Conclusion

Many potential local resources can be utilized as LWB to enhance soil fertility and improve plant growth, such as fish waste, seaweed, Azolla, fruit waste, *Moringa oleifera*, microalga, bamboo roots, banana hump, golden snail, mangrove leaves, fruit, and vegetable waste. Utilizing waste as LWB material can reduce the presence of organic waste in the environment. The challenges of implementing LWB in Indonesia include the availability of raw materials and raising awareness among Indonesian farmers to use LWB to achieve sustainable agriculture need to be addressed. Moreover, further research on adding compatible and efficient microorganisms to LWB formulas to enhance growth and yield is an interesting avenue for future research.

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Agronomic biofortification of calcium in pak choy (*Brassica rapa* subsp. *chinensis*) via hydroponic nutrient film technique

Abstract. Pak choy is rich in calcium, magnesium, phosphorus, iron, and vitamin K, all essential for health. Pak choy requires a proper balance of nutrients for optimal growth. In a hydroponic system, any imbalance in nutrient levels can negatively impact plant growth and reduce the nutritional value stored in the tissues. Selecting suitable varieties and providing appropriate calcium treatment can help increase calcium content and enhance productivity. This study aims to determine the effect of calcium concentration on growth, yield, and Ca content and the most responsive varieties to increased calcium concentration. The research design used was a Split Plot Design with two factors, namely calcium nitrate concentration (k) as the main plot consisting of k_0 = without addition of $Ca(NO_3)_2$, k_1 = addition of 300 mg/L $Ca(NO_3)_2$, k_2 = addition of 600 mg/L $Ca(NO_3)_2$, k_3 = addition of 900 mg/L $Ca(NO_3)_2$, and k_4 = addition of 1200 mg/L $Ca(NO_3)_2$. Varieties (v) as subplots consist of v_1 = Masbro, v_2 = Nauli F1, and v_3 = Flamingo. The results showed that the Nauli F1 variety had the best effect on the number of leaves, growth rate index, and fresh weight of shoots. Adding 900 mg/L of calcium nitrate had the best impact on plant growth, while adding 1200 mg/L of calcium nitrate caused plant poisoning. The Masbro variety was responsive to Ca biofortification.

Keywords: Biofortification · Growth · Macro nutrients · Toxicity

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Introduction

Brassicaceae vegetables are popular globally, with Pak choy (*Brassica rapa* subsp. *Chinensis*) being widely utilized in diverse culinary preparations worldwide. From Asian stir-fries to international fusion cuisine, peppery flavor and crunchy texture make it a favored ingredient. Pak choy is a highly nutritious vegetable, offering 13 kcal per 100 grams, with 95% of its weight coming from water. It also contains 1.5 grams of protein, 2.2 grams of carbohydrates, 0.2 grams of fat, 105 mg of calcium, 0.8 mg of iron, 19 mg of magnesium, 41 mg of phosphorus, and 252 mg of potassium.

The calcium given to pak choy benefits consumer health and plant growth and development. Calcium is a secondary macronutrient, although it is needed in smaller amounts compared to primary macronutrients. Calcium is used to control the pH of nutrient solutions. As the divalent cation (Ca^{2+}), it is required for structural roles in the cell wall and membranes, as a counter-cation for inorganic, and as an intracellular messenger in the cytosol (Ahmad et al. 2016). However, the calcium content in pak choy is relatively lower compared to other leafy greens like collard greens, which provide 232 mg of calcium per 100 g, and kale, which offers 150 mg per 100 g.

Efforts to increase the concentration of certain nutrients in plants by providing fertilizers can be achieved through agronomic biofortification (Farid & Ulinnuha, 2022). This process allows for the targeted accumulation of essential minerals, such as calcium, in plant tissues, enhancing their nutritional content. However, biofortification in conventional soil-based systems presents significant challenges. Soil reactions, interactions between nutrients, and the activity of microorganisms all influence the availability and uptake of minerals like calcium (Ceccherini et al. 2024). These complex factors make it difficult to control and optimize nutrient absorption in traditional agricultural settings, limiting the effectiveness of biofortification in soil-based systems. As a result, alternative approaches, such as hydroponic systems or more controlled soil management practices, may be necessary to achieve consistent and reliable nutrient enhancement in crops.

One of the popular hydroponic systems, namely the Nutrient Film Technique (NFT), can be defined as a type of closed-loop system that offers several advantages, particularly in biofortification. In an NFT system, a thin film of nutrient solution continuously flows over the plant roots, ensuring efficient nutrient uptake while minimizing water and nutrient wastage. This closed system significantly conserves nutrient availability, allowing for precise control over the concentration of essential elements, including those targeted for biofortification (Galić et al., 2021).

Agronomic biofortification using a hydroponic medium has been used several times, namely in the Zn biofortification of lettuce (de Lima et al., 2023), iodine on lettuce (de Figueiredo et al., 2022), iron on *Eruca sativa* L. (Ceccherini et al., 2024). Agronomic biofortification is expected to overcome nutritional deficiencies in various regions, improve public health, and provide better access to nutritious food. Also, agronomic biofortification can increase agricultural productivity and food security in the long term. So, this research aims to determine the effect of variety and calcium concentration on growth, yield, and Ca content in pak choy plant tissue.

Materials and Methods

Research activities have been conducted at the Screen House, Faculty of Agriculture, Jenderal Soedirman University, from December 2022 to March 2023. The materials in this research are Masbro pak choy variety, Nauli F1 pak choy variety, Flamingo pak choy variety, $Ca(NO_3)_2$, rockwool, water, plastic bucket, plastic tray, AB Mix, water pump, and NFT hydroponic installation. The tools used are a 1-liter measuring cup, knife, cutter, stationery, analytical scales, lux meter, thermo-hygrometer, spectrophotometer, and oven.

The experimental design used in this research was a Split Plot Design using two factors, namely calcium nitrate concentration as the main plot consisting of 5 levels, namely: k_0 = without addition of $Ca(NO_3)_2$, k_1 = addition of 300 mg /L $Ca(NO_3)_2$, k_2 = addition of 600 mg/L $Ca(NO_3)_2$, k_3 = addition of 900 mg/L $Ca(NO_3)_2$, k_4 = addition of 1200 mg/L $Ca(NO_3)_2$. Variety (v) as a subplot consists of 3 levels: v_1 = Masbro variety, v_2 = Nauli F1 variety, and v_3 = Flamingo variety. Combining the two factors resulted in 15 treatments with three replications, resulting in 45 research units. Each research unit consisted of two pak choy plants.

The parameters observed were plant height, number of leaves (pieces), leaf area (cm^2), root length (cm), root volume (mL), stem skin thickness (cm), growth rate index (g/week), fresh shoot weight (g), shoot dry weight (g), chlorophyll content, composite Ca content (%). The data obtained were analyzed using analysis of variance (ANOVA). If there were significant differences in the observed data, they were analyzed further using the Duncan Multiple Distance Test (DMRT) with a level of 5% and regression analysis on Ca content.

Results and Discussion

The following result in Table 1 is the recapitulation of the ANOVA result of numerous measured variables. Present study confirmed that the calcium treatment significantly affects all variables. This is in line with the research by Suryantini et al. (2020), which states that the addition of calcium nitrate significantly affects the growth and yield of curly lettuce plants. This indicates that the effect of calcium is significant on the development, physiology, and yield variables of pak choy plants.

Plant height. Plant height is an essential morphological and developmental phenotype that directly indicates overall plant growth and can predict plant yield and biomass (Wang et al., 2019). Calcium affects plant height, as presented in the following table (Table 2). Adding 900 mg/L of calcium nitrate produced higher plants up to 32.90 cm, but it was not significantly different with the addition of Ca at 0 and 300 mg/L. This research aligns with research (Suryantini et al., 2020), which states that the height of curly lettuce plants has increased due to the addition of $Ca(NO_3)_2$ with significantly different results. The element calcium plays a role in cell elongation and maintaining plant membrane structure (Zhang et al., 2020). Calcium-dependent protein kinases play a role in signal transduction pathways that regulate cell elongation in response to environmental cues (Yip et al. 2019). Cell elongation will produce taller plants (do Moraes et al. 2023).

Adding 1200 mg/L of calcium nitrate resulted in the lowest plant height 5 WAP was 26.13 cm. This could be an indication of calcium toxicity in pak choy plants. According to Reitz, et al. (2021), excessive calcium levels can disrupt the balance of other essential nutrients within the plant, such as potassium and magnesium. This imbalance can interfere with various physiological processes for plant growth and development.

Number of leaves. The number of leaves on a plant indicates the plant's performance. The statistical analysis shows an interaction between variety and calcium on the number of leaves at 5 WAP, which is presented in Table 3. The best interactions on the number of leaves variable, namely the Nauli F1 variety with the addition of 900 mg/L calcium nitrate (33.67 pieces) (Table 3). Environmental factors and genetic factors cause this interaction. The environmental factor in this research is the addition of calcium nitrate in hydroponic nutrients. Genetic factors play a role in plant responsiveness to the addition of calcium nitrate. In producing the number of leaves, the Nauli F1 variety was responsive to adding 900 mg/L of calcium nitrate. Plant responsibility can be seen from the calcium content data.

Table 1. Recapitulation of the Analysis of Variance (ANOVA) result of numerous measured variables as impacted by calcium concentrations, varieties and its combination.

No	Observation Variables	Variety	Calcium	Variety x Calcium
1	Plant height	ns	s	ns
2	Number of leaves	s	s	s
3	Leaf area (cm^2)	ns	s	ns
4	Root length (cm)	ns	s	ns
5	Root volume (ml)	ns	s	ns
6	Stem skim thickness (cm)	ns	s	ns
7	Growth rate index (g/week)	s	s	ns
8	Shoot Fresh Weight (g)	s	s	ns
9	Shoot Dry Weight (g)	ns	s	ns
10	Chlorophyll content (mg/g)	ns	s	ns

Note: ns: not significant; s: significant.

Table 2. Pak choy plant height in various varieties and concentrations of calcium addition.

Treatment factors	Plant height (cm)
Varieties	
Masbro (v_1)	29.09 a
Nauli F1 (v_2)	31.13 a
Flamingo (v_3)	30.09 a
CV (%)	7.27%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	30.51 a
300 mg/L (k_1)	30.71 a
600 mg/L (k_2)	30.15 ab
900 mg/L (k_3)	32.90 a
1200 mg/L (k_4)	26.13 b
CV (%)	7.27%

Note: Means on the same factor followed by the same letter are not significantly different based on the 5% DMRT.

Table 3. Number of pak choy leaves in various varieties and calcium concentrations.

Concentration of calcium nitrate addition	Masbro (V1)			Nauli F1 (V2)			Flamingo (V3)		
0 mg/L (k_0)	26.33	AB	a	27.67	BC	a	23.00	B	a
300 mg/L (k_1)	28.67	A	a	23.67	CD	b	20.00	BC	b
600 mg/L (k_2)	21.67	BC	b	30.33	AB	a	21.00	B	b
900 mg/L (k_3)	30.67	A	ab	33.67	A	a	28.33	A	b
1200 mg/L (k_4)	19.33	C	ab	22.33	D	a	15.33	C	b
CV (%)	11.31%			11.31%			11.31%		

Note: Means in the same column followed by the same capital letter are not significantly different based on the 5% DMRT; Means in the same row followed by the same lowercase letter are not significantly different based on the 5% DMRT.

Calcium (Ca) is a divalent cation and a vital element that plays a key role in maintaining the structure and permeability of cell membranes, as well as in processes such as plant cell division and elongation, carbohydrate transport, and nitrogen metabolism. During the early stages of leaf formation, an adequate calcium supply helps form new cells in the meristem (growth tissue), especially in the leaf primordia, which produces more leaves. Additionally, calcium is involved in regulating plant cell metabolism, signal transduction, and the uptake of nutrients through cell membranes (El-Beltagi & Mohamed, 2013).

Adding 1200 mg/L of calcium nitrate produced the lowest number of pak choy leaves, indicating calcium toxicity in pak choy plants. Calcium toxicity has side effects, including a deficiency of the Potassium nutrient. Potassium deficiency can hinder enzyme activity and plant photosynthesis (Divya et al. 2021). Sufficient potassium (K+) supply promotes photosynthetic assimilation, enhances nutrient uptake, and regulates leaf inclination by controlling turgor pressure. Potassium (K+) is essential in regulating stomatal opening, ensuring proper exchange of gases and water fluxes (Sardans & Peñuelas, 2021).

Leaf area. Leaf area is an essential indicator of a plant's photosynthetic capacity because it is directly related to the amount of light a plant can capture. The results of the DMRT test analysis on the effect of variety and calcium on leaf area are presented in Table 4.

Adding 300 mg/L calcium nitrate had a higher effect on leaf area, namely 85.33 cm^2, but not significantly different from adding 900 mg/L calcium nitrate, i.e., 82.56 cm^2. This is because the element calcium has a role in the formation, cell division, morphogenesis, and signaling in the formation and development of new plant organs (Himschoot et al., 2015). Calcium acts as a mitotic spindle and second messenger in the plasma membrane during the division process. Calcium is required to synthesize new cell walls, especially for the middle lamella. In the middle lamella, calcium forms electrostatic bonds with organic components (combining polygalacturonate with RCOO- groups (Huang et al., 2023). The membrane is maintained by calcium, which acts as a bridge between phosphate and carboxylate groups of phospholipids and surface proteins with the membrane (Melcrová et al., 2016).

Table 4. Leaf area of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Leaf area (cm)
Varieties	
Masbro (v_1)	71.89 a
Nauli F1 (v_2)	75.64 a
Flamingo (v_3)	78.94 a
CV (%)	23.13%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	74.92 ab
300 mg/L (k_1)	85.33 a
600 mg/L (k_2)	70.81 ab
900 mg/L (k_3)	82.56 a
1200 mg/L (k_4)	63.84 b
CV (%)	23.13%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Excessive calcium application can cause plant toxicity. This can be seen from the addition of 1200 mg/L $Ca(NO_3)_2$, which results in the lowest leaf area, i.e., 63.84 cm^2. Calcium toxicity can cause the absorption of magnesium (Mg) to be hampered. The Mg element plays a role in chlorophyll biosynthesis in plants. Hence, a decrease in Mg absorption in plants causes chlorosis, which decreases plants' photosynthesis rate (Gaj et al., 2018).

Stem skin thickness. The results of the variance analysis show that calcium influences stem skin thickness, which is presented in Table 4. Calcium treatment significantly affected stem skin thickness, as indicated by the addition of 900 mg/L calcium nitrate, which had the best impact, namely 0.12 cm, compared to other calcium treatments, which ranged from 0.08 - 011 cm. Wang et al. (2019) state that one of the benefits of calcium for plant growth is that it increases the development of plant leaves and stems. Calcium also affects cell elongation and division. Cell division and elongation will be directly proportional to the thickness of the stem skin obtained.

Adding 1200 mg/L calcium nitrate gave a lower result, namely 0.08 cm. This indicates the occurrence of toxicity in the pak choy plant. Nutrient toxicity is the level of a nutrient that can damage or be toxic to plants (Ahmad et al., 2016). Adding 1200 mg/L calcium nitrate produces lower stem thickness results than adding 900 mg/L calcium nitrate. This certainly affects the weight results that will be obtained later. High calcium content in the planting medium can harden plant cell walls, inhibit cell growth, disrupt phosphoric acid-based energy metabolism, and damage plant cytomembrane structures (Li et al., 2020).

Table 5. Stem skin thickness of pak choy in various varieties and concentrations of calcium addition

Treatment factors	Stem skin thickness (cm)
Varieties	
Masbro (v_1)	0.10 a
Nauli F1 (v_2)	0.09 a
Flamingo (v_3)	0.11 a
CV (%)	18.99%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	0.10 ab
300 mg/L (k_1)	0.11 ab
600 mg/L (k_2)	0.11 ab
900 mg/L (k_3)	0.12 a
1200 mg/L (k_4)	0.08 b
CV (%)	18.99%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Variety differences do not have a significant effect on stem skin thickness. The variety that produces the most considerable stem skin thickness is the Flamingo variety, with a stem skin thickness of 0.11 cm, but this is similar to other varieties, namely Masbro 0.10 cm and Nauli F1 0.09 cm. The thickness of the stem skin can be influenced by the variety used. Each variety has different genetic characteristics. This genetic trait influences stem thickness.

Root Length and Root Volume. Root length and volume are influenced by increasing calcium concentration. The results of the DMRT test analysis on the effect of variety and calcium on root length and root volume are presented in the Table 6.

The DMRT results stated that 900 mg/L calcium nitrate treatment gave better results in root volume, i.e., 40.89 ml, and root length, i.e., 49.47 cm. An increase in root length provides the most optimum nutrient availability for pak choy plants. Calcium ions regulate the flux of other ions, such as potassium and chloride, across the cell membrane. This ion flux is crucial for maintaining cellular osmotic balance, cell expansion, and turgor pressure regulation (Kashtoh & Baek, 2021).

Adding 1200 mg/L calcium nitrate resulted in the lower root volume, i.e, 24.44 ml, and the lowest root length, i.e., 25.84 cm, in pak choy plants. This indicates that adding calcium nitrate that exceeds the optimum point for pak choy plant nutrition can reduce root volume and length because genetic diversity in root architecture may be limited.

Table 6. Root length (cm) and root volume (ml) of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Root length (cm)	Root volume (ml)
Varieties		
Masbro (v_1)	31.49 a	26.67 a
Nauli F1 (v_2)	37.79 a	32.93 a
Flamingo (v_3)	42.03 a	28.93 a
CV (%)	24.23%	24.06%
Concentration of calcium nitrate addition		
0 mg/L (k_0)	35.19 ab	24.89 b
300 mg/L (k_1)	41.47 ab	26.67 b
600 mg/L (k_2)	33.54 ab	30.67 ab
900 mg/L (k_3)	49.47 a	40.89 a
1200 mg/L (k_4)	25.84 b	24.44 b
CV (%)	24.23%	24.06%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Growth Rate Index. The results of the DMRT test analysis on the effect of variety and calcium on the growth rate index are presented in Table 7. Adding calcium nitrate 900 mg/L gave the best effect of 131.44 g/week. Providing calcium nitrate fertilizer can increase the absorption of calcium and nitrate, as well as increase plant yields (Seyfferth & Tsuda, 2014). The calcium element itself has a role in improving plant production. The growth rate index is based on the power value of the growth rate based on plant weight over time. The growth rate index will also be high if the plant weight is high. So, providing optimum calcium fertilizer for plants can increase the growth rate index.

Adding 1200 mg/L resulted in the lowest growth rate index of 31.28 g/week. This can be caused by giving too much calcium nitrate fertilizer, inhibiting pak choy plants' growth. According to Aryandhita & Kastono, (2021), excess calcium can inhibit plant production. The growth rate index is related to the fresh weight of the plant, so if production is not optimal, the growth rate index will be smaller.

Table 7. Growth rate index of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Growth rate index (g/week)
Varieties	
Masbro (v_1)	65.29 b
Nauli F1 (v_2)	86.05 a
Flamingo (v_3)	68.57 b
CV (%)	21.19%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	70.02 b
300 mg/L (k_1)	67.28 b
600 mg/L (k_2)	66.51 b
900 mg/L (k_3)	131.44 a
1200 mg/L (k_4)	31.28 c
CV (%)	21.19%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

Variety treatment had a significant effect on the growth rate index. The variety that had the best influence on the growth rate index was the Nauli F1 variety, with an index value of 86.05 g/week. This is done by describing the varieties of each pak choy plant. In the description of plant varieties, the Nauli F1 variety has the highest productivity compared to other varieties, namely 37 – 39 tonnes/ha or 400 – 500 g/plant. This productivity is, of course, in line with the growth rate index, which is related to the fresh weight of the plant canopy. The greater the fresh weight of the canopy, the higher the growth rate index.

Shoot Fresh Weight and Shoot Dry Weight. The results of the DMRT analysis on the effect of variety and calcium on shoot fresh weight and shoot dry weight are presented in Table 8. Adding 900 mg/L calcium nitrate had the best effect on the variable fresh weight (364.15 g) and dry weight of the pak choy plant canopy (10.67 g). This is in line with research by Suryantini, et al. (2020), which stated that the addition of 90 g of calcium nitrate fertilizer (in 100 L) was able to increase production results because the availability of the nutrients provided was in a balanced state and by the needs of curly lettuce plants.

Table 8. Shoot fresh and dry weight of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Shoot fresh weight (g)	Shoot dry weight (g)
Varieties		
Masbro (v_1)	178.99 b	6.74 a
Nauli F1 (v_2)	257.96 a	8.53 a
Flamingo (v_3)	178.99 b	7.50 a
CV (%)	22.27%	23.00%
Concentration of calcium nitrate addition		
0 mg/L (k_0)	207.60 b	7.60 ab
300 mg/L (k_1)	197.42 b	7.07 ab
600 mg/L (k_2)	204.59 b	7.75 ab
900 mg/L (k_3)	364.15 a	10.67 a
1200 mg/L (k_4)	94.80 c	4.86 b
CV (%)	22.27%	23.00%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT.

The high fresh weight of the canopy is caused by the relatively high leaf area and number of leaves. In the leaf area analysis results, adding 300 mg/L Ca treatment produced the highest leaf area, 85.33 cm^2. However, this was not significantly different from the leaf area of the additional 900 mg/L Ca treatment, namely 82.56 cm^2. In the analysis of the number of leaves, the addition of 900 mg/L Ca treatment produced a higher number of leaves, namely 30.89, compared to the addition of 300 mg/L Ca treatment, namely 24.11. So, if the two variables are correlated, they are related to the fresh weight of the shoot produced.

In leaf vegetable commodities, an increase in the number of leaves will result in a higher fresh shoot weight. The wider the plant leaves, the wider the area to capture light processed in photosynthesis. The photosynthesis process increases with the increasing plant growth rate (Wang et al., 2019). When adding 1200 mg/L, fresh weight decreased due to the addition of excess calcium nitrate. The addition of excessive concentrations of calcium elements can inhibit the absorption of K or Mg elements. Both elements play a role in photosynthesis (Weng et al., 2022). Adding 1200 mg/L calcium nitrate resulted in the lowest dry weight of pak choy shoots. Plant dry weight reflects the nutritional status of the plant. Research conducted by Aryandhita & Kastono, (2021) stated that increasing calcium concentration can increase plant dry weight because adding calcium nutrients streamlines the plant's ability to absorb and use photosynthate. However, adding excess calcium can also inhibit plant growth and cause a decrease in yield.

The Nauli F1 variety had the best effect on the shoot's fresh weight, which was significantly different than others. Based on the description of Masbro, Nauli F1, and Flamingo varieties, it can be seen that Nauli F1 has the highest productivity compared to other varieties. The results of this research also align with the description of the variety, namely that the Nauli F1 pak choy variety has the highest productivity compared to other varieties.

Chlorophyll content. The results of the DMRT test analysis on the effect of variety and calcium on chlorophyll content are presented in Table 9. Adding 0 mg/L calcium nitrate was 15.51 mg/L chlorophyll content, significantly different from other calcium treatments. Chlorophyll is the main factor that influences photosynthesis. Photosynthesis is the process of changing inorganic compounds (CO_2 and H_2O) into organic compounds (carbohydrates) and O_2 with the help of sunlight (Ardiansyah et al., 2022).

Table 9. Chlorophyll content of pak choy in various varieties and concentrations of calcium addition.

Treatment factors	Chlorophyll content (mg/L)
Varieties	
Masbro (v_1)	9.88 a
Nauli F1 (v_2)	10.86 a
Flamingo (v_3)	11.14 a
CV (%)	22.73%
Concentration of calcium nitrate addition	
0 mg/L (k_0)	15.51 a
300 mg/L (k_1)	9.31 bc
600 mg/L (k_2)	9.44 bc
900 mg/L (k_3)	12.49 ab
1200 mg/L (k_4)	6.39 c
CV (%)	22.73%

Note: Numbers on the same factors and variables followed by the same letters are not significantly different according to the 5% DMRT Test.

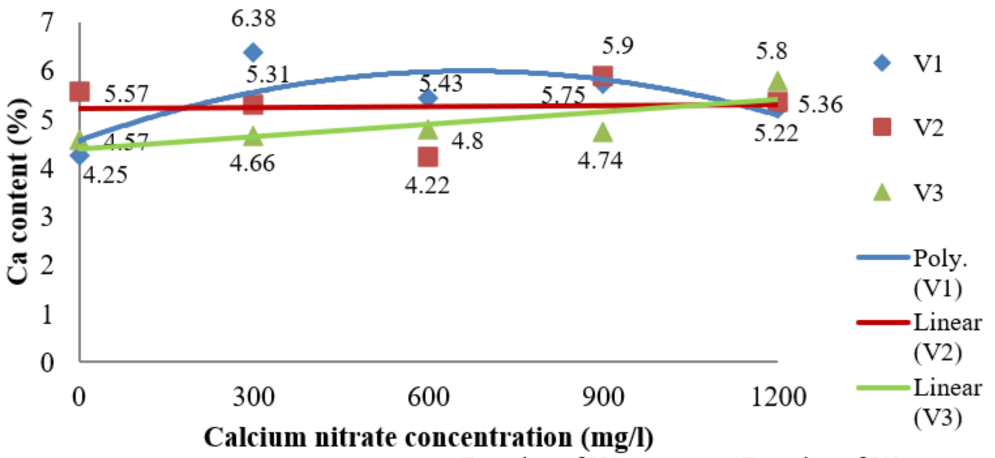
The addition of calcium nutrients through fertilization has side effects in the form of deficiencies in the nutrients K and Mg when the Ca content is high in plants. K deficiency in plants can disrupt the photosynthesis process and plant enzyme activity (Aryandhita & Kastono, 2021). Photosynthesis is what will affect the chlorophyll content in plants. There is no diversity of chlorophyll in the varieties used. According to

Wang et al., (2023b), chlorophyll biosynthesis is carried out by specific genes in the chromosomes. This gene encodes an enzyme that will play a role in the biosynthesis pathway of tetrapyrrole (porphyrin nucleus) as the structural center of chlorophyll.

Calcium content. Calcium is a secondary macronutrient that is important in plant growth and development. Calcium is part of plant cell walls and plays a role in various metabolic processes, including activation of enzymatic systems, membrane stability, and cell integrity. The following regression graph presents the influence of variety and calcium on Ca content (Figure 1).

The results of the quadratic regression analysis (Figure 1) on V1 obtained the equation y= -3E -06x2 + 0.0043x + 4.5654. The optimum x value obtained from the quadratic equation is 716.67. So, the best calcium treatment based on the regression graph is between the 600 mg/L and 900 mg/L calcium nitrate treatments in V1. The R^2 value = 0.5508h indicates that calcium has a moderate influence on the Ca content of the Masbro variety. The results of the quadratic y= 6E-05x + 5.238. The graph shows that the linear line tends to be flat but is still increasing, even though it is small. The highest point of the linear line is at a calcium concentration of 1200 mg/L. The R^2 value = 0.0018 indicates that calcium weakly influences the Ca content of the Nauli F1 variety. The results of the quadratic regression analysis on V3 obtained the equation y = 0.0008x + 4.406. The graph shows that the linear line tends to rise and is at its highest point when the calcium concentration is 1200 mg/L. The R^2 value = 0.6381 indicates that calcium has a moderate influence on the Ca content of the Flamingo variety.

The variety that produces the highest Ca content is the Masbro variety, while the variety that produces the lowest is the Nauli F1 variety. In the variety description, the Nauli F1 variety has the highest productivity but has the lowest Ca content compared to other varieties. This can be indicated because the calcium absorption responsibility of each variety is different. The genetic characteristics of each plant variety cause this responsibility. So, even though the Nauli F1 variety has the highest productivity in the description, its responsibility for calcium nutrients is low. Different plant varieties may have inherent genetic differences that influence their ability to absorb and accumulate nutrients. Genetic variation can affect traits such as root morphology, nutrient uptake kinetics, nutrient transporters, and nutrient storage mechanisms (Griffiths et al., 2021).The study showed that adding 900 mg/L of calcium nitrate positively impacted plant growth. Specifically, the treatment led to an increase in both the number and the area of the leaves. As the number of leaves increased, the plant's ability to carry out photosynthesis was significantly enhanced. This improvement can be attributed to the fact that each additional leaf contributes to a larger overall surface area, which allows the plant to capture more sunlight. The plant can absorb more light with more leaves, increasing the production of energy-rich compounds (Zhou et al. 2023). This increased photosynthetic capacity supports faster growth and helps the plant accumulate more resources for development, ultimately improving overall plant growth.



Equation of V1	Equation of V2	Equation of V3
$y = -3E\text{-}06x^2 + 0.0043x + 4.5654$	$y = 6E\text{-}05x + 5.238$	$y = 0.0008x + 4.406$
$R^2 = 0.5508$	$R^2 = 0.0018$	$R^2 = 0.6381$

Figure 1. Ca Content of pak choy tissue in various varieties and Ca addition.

Zahro RS, Faozi K, Ulinnuha Z, Sarjito A. 2024. Agronomic biofortification of calcium in pak choy (*Brassica rapa* subsp. *chinensis*) via hydroponic nutrient film technique. Jurnal Kultivasi, 23(3): 291-301.

There was an increase in plant growth rate with the addition of 900 mg/L of calcium nitrate. As a result of this increased growth, the plants also showed a substantial increase in fresh weight. Additionally, the accumulation of photosynthates, sugars, and other organic compounds produced during photosynthesis was evident in the plant's dry weight. This suggests that the plants increased growth and became more efficient in storing energy, as evidenced by the increased accumulation of solid biomass. As the plants grow faster, they expand in size and devote more resources to developing structural components, such as roots, stems, and leaves. These structures store energy in the form of carbohydrates and other organic compounds produced during photosynthesis. The enhanced storage of energy in the form of solid biomass indicates that the plants could convert more of the absorbed nutrients into usable, long-term reserves. The study's findings show the significant role that calcium nitrate plays in supporting plant development. Providing calcium compounds can appear to stimulate growth processes and promote better nutrient utilization (Weng et al., 2022).

Although a 900 mg/L calcium nitrate increase significantly increased growth, it did not increase calcium accumulation in plant tissues. This is related to Calcium primarily functioning as a structural element in plant cells. Calcium stabilizes cell walls by forming pectate complexes in the middle lamella, which connect plant cells. Calcium regulates membrane structure and function and is a secondary messenger in signal transduction processes (Thor, 2019). Thus, calcium is not stored in large amounts like other nutrients but is incorporated into cellular structures and used in signaling. Although more calcium may lead to increased growth due to better cell wall structure and more vital cell membranes, the total accumulation of calcium in tissues may not increase significantly because plants use it for specific structural and regulatory roles rather than storing it in large amounts (Wdowiak et al., 2024).

Conclusion

Adding 900 mg/L calcium nitrate had the best effect on almost all variables except leaf area, chlorophyll content, and composite Ca content. However, the addition of 1200 mg/L calcium nitrate was indicated to be toxic to the growth of pak choy. The Masbro variety was responsive to increasing Ca content in the tissue, but the Nauli F1 and Flamingo varieties were not responsive to CA addition.

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Potential propagation seedling of ramie (*Boehmeria nivea*) from various types of stem cuttings

Abstract. Ramie is a fiber-producing plant that can replace cotton as a raw material for the textile industry. In general, ramie plants are reproduced using rhizomes, but it takes a long time, around two years, to be used as a source of planting material. Therefore, other sources of explants besides rhizomes are required, such as stem cuttings. Explants from stem cuttings are quickly available and only need 2-3 months to be used as planting material. This study aims to determine the potential of effective stem cuttings that can be used as planting material for the propagation of ramie seedlings. The research was conducted from July to December 2021 at the experimental garden in the Indonesian Instruments Standardization Testing Center for Sweetener and Fiber Crops, Malang, East Java. The research used a Completely Randomized Design (CRD) consisting of four treatments of ramie seedling material of the Ramindo 1 variety, namely rhizome, shoot cuttings, middle stem cuttings, and basal stem cuttings. Each treatment was repeated 5 times. The result showed that all parameters had a significant effect. Ramie seedling source from basal stem cuttings showed the best growth percentage (92%), stem diameter (4.8 mm), and plant wet weight (44.6 g). Shoot cuttings showed the best number of roots (31.6), plant height (60.8 cm), and number of leaves (41.4). Cuttings from shoot and basal stems can be used as planting material for producing ramie seeds other than rhizomes.

Keywords: Basal stem · Natural fiber · Ramindo 1 · Rhizome

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Introduction

Natural fibers are widely developed in many countries, including Indonesia, which can produce biomass such as jute, flax, cotton, abaca, sisal, kenaf, and ramie, which may be biodegradable. (Elfaleh et al., 2023). Although ramie plants have long been developed in China since 5000-3000 BC (Sen & Reddy, 2011), they have also been widely developed in Japan, the Malay Peninsula (Bayan et al., 2018), India, and Indonesia (Roy & Lutfar, 2020). Ramie fibers are extensively used in the textile industry. (Ullah et al., 2016; Bakshi et al., 2024). Ramie stems can be turned into paper pulp, biocomposites, catalysis, sound absorption, biomedical materials, and fuel. (Sharma et al., 2014; Zhong et al., 2020; Cho et al., 2022), leaves can be used as compost and high-nutrient animal feed (Tang et al., 2021), and rhizomes (roots) are used as planting materials (Nuraini et al., 2022).

The development of ramie to support the textile industry and other textile products has not been optimal due to the lack of use of the superior Ramindo 1 variety. This variety has the advantage of a high fiber yield (Maideliza et al., 2017) and high fiber productivity, reaching 2-2.7 tons/ha/year (Novarini & Sukardan, 2015). Additionally, the availability of sufficient and high-quality seeds is still very limited. Meanwhile, the need for ramie per hectare requires seeds with a minimum standard of 20000 - 25000 rhizomes per hectare. Currently, ramie development is limited to Central Java, West Java, Lampung, and South Sumatra, so the expansion of planting areas needs to be optimized. A sustainable supply of ramie seedlings is needed to support this. However, it is not in line with the current ramie seedling supply. This is because ramie seedlings can only be provided through conventional methods using rhizomes (Nuraini et al., 2022) from plants that are more than 2 years old. A rhizome is a part of the plant located below the soil surface, serving a different function than roots or radicles, and has horizontal growth (Genosko, 2020). So, producing ramie seedlings takes a long time and the rhizomes cannot be stored for long.

One of the efforts to propagate ramie seedlings can be done vegetatively by utilizing the plant stem cuttings. Ramie is a perennial plant that can be harvested every 60 days by cutting the base of the stem without uprooting the plant (Subandi, 2012). The appropriate stem-cutting method can produce seedlings at a low cost, with high viability, in large quantities, and uniformly (Apriani & Suhartanto, 2015).

Propagation of seedlings through stem cuttings can use the stem from the shoot, stem cuttings from the middle stem, and stem cuttings from the basal stem. The selected stems should have a uniform diameter and have turned brown (Bayan et al., 2018). It is hoped that all types of stem cuttings have the potential to be used as seedling sources of the same quality as rhizomes so that no part of the ramie plant stem is wasted.

The previous studies by Suherman, et al. (2016; 2017) often used rhizomes cut to a length of 10-15 cm from the Ramindo 1 variety in the propagation and production of ramie. Using stem cuttings from the shoot with a length of 7 cm is the best stem cutting that can be used as planting material. Shoot cuttings can produce vigorous *Plectranthus amboinicus Spreng* stem cuttings based on the success components of the cuttings and their growth (Apriani & Suhartanto, 2015). Stem cuttings from the basal stem can provide the best number of new leaves and root fresh weight in *Alstonia scholaris* plants, although not significantly different from other treatments (Putri et al., 2018). This study aims to determine the potential of effective stem cuttings that can be used as planting material for the propagation of seedlings of ramie.

Materials and Methods

Plant Material. The experiment was conducted at the experimental garden in Indonesian Instruments Standardization Testing Center for Sweetener and Fiber Crops, Malang, East Java, from July to December 2021. The ramie plants used were of the Ramindo 1 variety, which was 2 years old. Rhizome collection was carried out by digging up the roots of the ramie plants and washing them with water until clean. The planting material used for stem cuttings is rhizomes obtained from the root part of the plant that is 2 years old, characterized by having straight stems, visible buds, and a dark brown color with a diameter of about 0.5–0.8 cm and a rhizome length of 10 cm. Stem cuttings from the tip, middle, and base parts are obtained from ramie stems that are 2 months old, with a diameter of 0.3–0.5 cm and a stem length of 10 cm. The base stem cuttings have the criteria of a dark brown stem, the middle stem cuttings have a brownish-green color, and the tip cuttings are characterized by having growth points

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and retaining 2–3 leaves that are still growing (Figure 1).

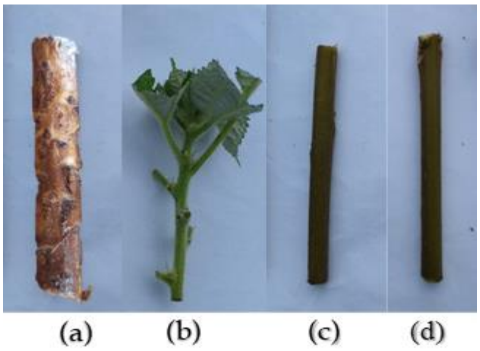

Figure 1. Types of ramie stem cuttings whose basic explant sources come from: propagation from rhizomes (a), propagation from shoot stems (b), propagation from middle stem cuttings (c), and propagation from base stem cuttings (d).

Seedling sowing. The planting materials are collected and selected according to the criteria for the stem-cutting treatment used. Each type of stem cutting was sown in pot trays filled with a growing medium consisting of soil, compost, and mixed rice husk charcoal in a ratio of 1:1:1. Before planting, the stem cuttings were dipped in a *Rootone F* solution at a dose of 20 g per L for 5 minutes as a root primordia-stimulating hormone.

The pot trays sowed with ramie stem cuttings were then covered with a plastic polyethylene transparent cover to maintain temperature (20-30°C) and humidity (60 – 80%). The maintenance during the ramie cuttings nursery included watering, weeding, and pest control by spraying fungicides with the active ingredient *Mancozeb* at 2 g per L and insecticides with the active ingredient *Carbosulfan* at 5 g per L.

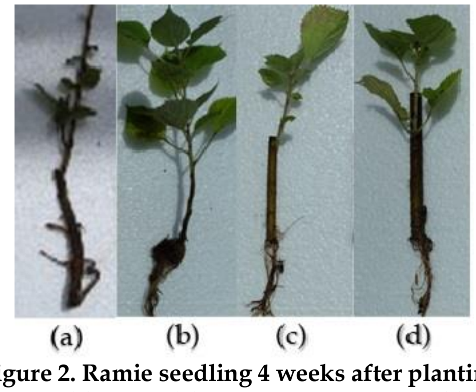

Figure 2. Ramie seedling 4 weeks after planting: rhizome (a) Shoot (b), middle stem cutting (c), and basal stem cutting (d).

Seedling planting. Transplanting was carried out when the ramie seedlings were 4 weeks old (Figure 2) into polybags measuring 20 cm x 20 cm filled with a growing medium consisting of a 1:1 mixture of soil and rice husk charcoal. Transplanting was done in the afternoon, and the ramie plants were watered until the polybags reached field capacity.

Plant maintenance included watering, manual weeding, and pest and disease control by spraying fungicides with the active ingredient *Mancozeb* at 2 g per L and insecticides with the active ingredient *Carbosulfan* at 5 g per L in 6, 8, and 10 weeks after planting (WAP). Fertilization was also carried out by applying NPK Mutiara fertilizer at 3 grams per plant at 2 and 6 WAP.

Observations. Observations were made on the growth percentage (%) by counting the number of seedlings that grow, dividing it by the number of seedlings planted, and multiplying by 100% at 4 weeks after planting (WAP), shoot length (cm) at 4 WAP, while plant height (cm), number of leaves, root length (cm), number of roots, stem diameter (mm), and plant fresh weight (g) were observed at 12 WAP.

Data analysis. The experimental design used a Completely Randomized Design (CRD) with a single factor, which was the type of stem cutting consisting of four types: rhizome, shoot cuttings, middle stem cuttings, and basal stem cuttings. Each treatment was repeated 5 times, resulting in 20 experimental units. Data were analyzed using analysis of variance (ANOVA) with SAS version 9.4 for Windows (SAS Institute Inc, 2023). The significant difference in the mean value of the treatment was further tested using Tukey's Honestly Significant Difference (HSD) at a significant level of 5%.

Results and Discussion

Growth percentage and shoot length. Plant growth can begin with germination, marked by the emergence of the radicle, which produces roots, and the plumule, which forms shoots. The germination process affects the survival rate of seedlings and plant growth, which is also influenced by the climate (Haj Sghaier et al., 2023). The treatment of different types of ramie stem cuttings showed a significant effect on the growth percentage of ramie stem cuttings (Table 1). Ramie stem cuttings from the basal stem showed the highest growth percentage, reaching 92.4%, but this was not significantly different from the rhizome cuttings, which had a growth percentage of 87.8%.

Table 1. Effect of stem cutting types on growth percentage and shoot length ramie seedlings

Type of stem cuttings	Growth percentage (%)	Shoot length (cm)
Rhizome	87.8 ab	15.0 a
Shoot cuttings	85.8 b	11.0 b
Middle stem cuttings	80.4 c	11.0 b
Basal stem cuttings	92.4 a	7.2 c
Mean	86.6	11.1
Coefficient variance	3.9	13.9
P-value	0.0009**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

The high growth ability of ramie seedlings originating from the basal stem indicates that the basal stem has more mature tissues ready to support root growth compared to the upper or middle stem cuttings. The basal stem cutting also often has more carbohydrate reserves and nutrients, such as hormones that can stimulate the growth of new shoots and roots. This is consistent with the findings of Husen (2012), which stated that carbohydrate reserves in the stem promote rooting when combined with effective auxin treatment. Additionally, the average germination percentage of seedlings reached 86.6%, categorizing ramie stem cuttings as high-quality seeds. On the whole, a seed germination percentage rate above 80% is considered quite high and indicates superior seed quality (Bobokalonov & Cheryomushkina, 2018). This result also aligns with the research by Lodhiyal, et al. (2023) which found that vegetative propagation of some plants through stem cuttings showed a success rate of over 80%.

The type of ramie cutting showed a significant effect on the shoot length of ramie at 4 WAP. Stem cutting using rhizomes showed the best shoot length, reaching 15 cm, which was significantly different from the other stem cutting treatments. The shoot length resulting from stem cuttings can significantly affect the growth and development of the plant. Cuttings from longer rhizomes usually have more energy reserves in the form of starch, which can form shoots and elongate roots. Similarly, highland plants can store fructans as carbohydrate reserves in their roots during winter to produce tuna in the following season (Yoshida, 2021). The photosynthesis results obtained by each plant will affect the amount of photosynthesis in the form of carbohydrates. These carbohydrates are also abundant in rhizomes, where the rhizome is the main factor influencing shoot development (Suherman et al., 2017).

Plant height and number of leaves. Table 2 shows that the type of ramie stem cutting has a significant effect on plant height and the number of leaves. Shoot cuttings showed the highest plant height, reaching 60.8 cm, but this was not significantly different from the middle stem and basal stem cuttings. The same pattern was observed in the number of leaves, with shoot cuttings having the highest number of leaves, 41.4 leaves, which was significantly different from the other stem-cutting types.

Increased plant height and the number of leaves can have a significant impact on the overall growth of ramie plants. The highest plants with more leaves often have better access to light and can utilize more nutrients from the growing medium. According to Tanaka et al., (2006) in *Pisum sativum L*, auxin plays a role in inhibiting cytokinin biosynthesis in the nodal stem to suppress the growth of axillary buds and enhance apical dominance. Auxin is the main hormone that controls the occurrence of apical dominance, primarily playing a role in the long-distance polar auxin transport system and local auxin biosynthesis in modulating shoot branching (Thelander et al., 2022). The research by Apriani & Suhartanto (2015) showed that shoot cuttings also produced the best plant height and number of shoots compared to cuttings from the middle and basal stems in *Plectranthus amboinicus Spreng* plants.

Root length and number of roots. The plant's ability to absorb nutrients and water can be determined by root growth. The elongation of plant cells, involving the cell wall and turgor pressure on the cell wall, can lead to root emergence. The ability of leaves to photosynthesize actively can influence the length and number of plant roots (Rayburn & Sharpe, 2019; Ye et al., 2023). The type of stem-cutting treatment showed a significant effect on root length and the number of roots (Table 3).

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Table 2. Effect of stem cutting types on plant height and number of leaves ramie seedlings.

Type of stem cuttings	Plant height (cm)	Number of leaves
Rhizome	43.8 b	29.4 c
Shoot cuttings	60.8 a	41.4 a
Middle stem cuttings	58.0 a	30.4 bc
Basal stem cuttings	60.2 a	33.8 b
Mean	55.7	33.7
Coefficient variance	3.9	8.1
P-value	<.0001**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

Table 3. Effect of stem cutting types on root length and number of roots ramie seedlings.

Type of stem cuttings	Root length (cm)	Number of roots
Rhizome	11.0 a	30.4 a
Shoot cuttings	4.2 b	31.6 a
Middle stem cuttings	5.4 b	12.8 b
Basal stem cuttings	5.2 b	15.2 b
Mean	6.5	22.5
Coefficient variance	23.0	19.0
P-value	<.0001**	<.0001**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

Rhizome cuttings showed the best root length, which was 11 cm, and this was significantly different from the other stem-cutting treatments. Meanwhile, for the number of roots parameter, the shoot cuttings showed the highest number of roots, reaching 31.6, but this was not significantly different from the rhizome cuttings.

A rhizome is a horizontally oriented stem (some species have vertical positions) that grows underground and can produce roots and shoots at its nodes. Rhizomes function as nutrient storage for the vegetative propagation of the parent plant and the distribution of plant species (Li et al., 2022; Stedeford, 2023; Petruzzello, 2024). The root length and number of roots in stem cuttings derived from rhizomes are quite good compared to other stem cuttings because the surface of the rhizome has many thin meristematic tissues capable of forming roots. According to Guo, et al. (2021), rhizomes have apical meristem tissues with thick epidermal surfaces to protect the tissues and aid in pushing through the soil. The nodes on ramie rhizomes produce additional roots to expand the plant's root system.

The roots of shoot cuttings are fibrous and have younger tissues, making it easier for roots to emerge and resulting in a higher number of roots than other stem cuttings. The research by Apriani & Suhartanto (2015) showed that stem cuttings taken from the shoot had better root systems in *Plectranthus amboinicus Spreng* plants compared to cuttings taken from the middle and basal stem. In *Gyrinops versteegii* plants, the best rooting was also observed in shoot cuttings with soil media and the use of IBA at a concentration of 200 ppm (Setyayudi, 2018).

Stem diameter and plant fresh weight. A uniform stem diameter can provide better structural stability and support yield production without the risk of damage. Ohta & Makino (2019) stated that a uniform and upright stem diameter can enhance plant production in supplying fruit to meet market demand. The type of cutting treatment showed a significant effect on increasing the stem diameter of ramie plants. Stem cuttings from the basal stem provided the highest stem diameter, which was 4.8 mm, although it was not significantly different from the other treatments except for the rhizome. The basal stem has a slightly larger diameter compared to other stem cuttings. The research by Suherman, et al. (2016) showed that the average stem diameter of Ramindo 1 ranged from 34-53 mm at 12 WAP. The length and diameter of stem cuttings can affect the efficiency of rooting emergence (Lebedev, 2019). Producing the best stem cuttings is also influenced by the cutting method and the diameter of the stems used (Kumar et al., 2023).

Table 4. Effect of stem cutting types on Stem diameter and plant fresh weight ramie seedlings.

Type of stem cuttings	Stem diameter (mm)	Plant fresh weight (g)
Rhizome	4.2 b	37.8 b
Shoot cuttings	4.6 ab	38.8 b
Middle stem cuttings	4.6 ab	39.4 b
Basal stem cuttings	4.8 a	44.6 a
Mean	4.6	40.2
Coefficient variance	7.2	5.8
P-value	0.0087**	0.0027**

Note: Mean followed by the same lowercase alphabet in the same column is not significantly different based on Tukey's Honestly Significant Difference (HSD) test at the level of 5 %.

The high fresh weight of the plant indicates greater biomass production and correlates with increased yield obtained from the photosynthates produced and allocated for the growth of fruit or seeds. Du, et al. (2021) stated that the fresh weight and dry weight of the plant can illustrate the overall accumulation of plant biomass and serve as good indicators of plant growth. Table 4 shows that the type of stem cuttings has a significant effect on the fresh weight of the plant. Stem cuttings from the basal stem showed the best plant fresh weight, which was 44.6 g, and this was significantly different from other types of cuttings.

The larger the stem diameter, the higher the plant's fresh weight, and this relationship is linear. The potential plant's fresh weight derived from basal stem cuttings tends to be higher because the diameter of the basal stem gradually decreases from the lower base toward the top of the plant. The level of plant fresh weight accumulation in each stage of plant growth shows an increasing trend (Bai et al., 2020). This is thought to be due to the presence of organic or chemical substances in plant tissue. In the chemical analysis of fiber, 71.75% cellulose, 12.11% hemicellulose, 1.06% lignin, and 1.70% ash were found (Marinho et al., 2018). Research by Suherman et al. (2017) showed that Ramindo 1 had the best fresh stem and leaf weight and was not significantly different from Bandung A clone ramie. Research by Putri, et al. (2018) also showed that stem cuttings from the base of the stem had a high fresh root weight in Alstonia Scholaris and were not significantly different from other treatments.

Conclusion

The type of stem cutting from the basal stem showed the best growth percentage (92.4%), stem diameter (4.8 mm), and plant fresh weight (44.6 g). Shoot cuttings showed the best number of roots (31.6), plant height (60.8 cm), and number of leaves (41.4). Cuttings from shoot and basal stems can potentially be used as planting material for the production of ramie seeds other than rhizomes.

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Prediction of NPK doses based on targeted fruit sugar content in *Cucumis melo* L. 'Cantaloupe' using a simple regression method

Abstract. The fruit sweetness is the main target in melon plant production. The highest criterion of sweetness is excellent, with 16% of total sugar content. Modification of essential plant nutrients is the alternative to reach that category. So, this study aims to obtain optimum NPK doses using a simple regression method. The experiment was conducted in a greenhouse with a soilless culture hydroponic system from August until November 2023 using a completely randomized design (CRD) with five treatments and four replications. The parameters included leaf area, plant dry matter, leaf nutrient uptake, fruit weight, and fruit sugar content. Pearson correlation analysis showed that the total sugar content in fruit has a significantly positive correlation with potassium in NPK fertilizer treatments such as K_2O dose and K_2O uptake at 7 WAP, i.e., 0.932 and 0.973, respectively. According to the regression model y = -50.7 + 1.079 N + 0.251 P_2O_5 + 0.528 K_2O, the NPK formula fertilizer containing 31.56 g N, 23.99 g P_2O_5, and 50.42 g K_2O can be used by grower to produce excellent fruit sugar content.

Keywords: Melon · NPK · Potassium · Regression · Sugar

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Introduction

Melon (*Cucumis melo* L.) included a horticulture plant with fruit as an organ target. In Indonesia, the productivity of melon from 2018 until 2022 was 119 tons, 122 tons, 138 tons, 129 tons, and 119 tons, respectively (Statistic Indonesia, 2023). Food and Agriculture Organization (FAO) reported worldwide productivity of melons reached more than 28 million tons in 2020 (Xu et al., 2022). The data indicated an increasing number of people choosing melons to supply the demand for fruit. Manchali et al. (2021) stated that shape, size, taste, aroma, skin, and flesh color are criteria for consumers' acceptance of melon fruit. Indonesians prefer melon, which has characteristics such as large fruit size (2-3 kg), strong fruit aroma, crunchy flesh texture, high fruit sweetness (^{0}Brix 12-13), and relatively long shelf life of ±10 days (Khairi et al., 2017). In other countries, such as Taiwan, consumers prefer small size (1-2 kg), distinctive aroma, high fruit sweetness (^{0}Brix 13-14), and crunchy texture (Yam et al., 2020). Some reports said that the priority criterion is sweetness.

Adams (2023) stated that the sweetness quality criteria of 'Cantaloupe' melons by the refractive index of crop juices, including poor (8%), average (12%), good (14%), and excellent (16%). Hydroponic system melon has sweeter fruit than open-field melons. Genotype, light, temperature, humidity, water supply, nutrient composition, and pH of the nutrient solution affected melon production in the hydroponic systems (da Silva et al., 2019). Yam et al. (2020) reported that nutrient composition directly enhances the quality of melon fruit sweetness in a hydroponic system. Specifically, potassium positively correlates with melon fruit's increasing sugar content, as Rangel et al. (2018) reported. In hydroponic systems, Johnson & Mirza (2020) stated that plants are required to supply essential nutrients, such as nitrogen (N), phosphate (P), and potassium (K), to complete the plant life cycle optimally.

The determination of NPK formulation was reported using a mathematical regression model. Suminar (2017) recommends NPK doses of about 160.4 Kg/ha N, 43.7 Kg/ha P_2O_5, and 124.9 Kg/ha K_2O for sorghum production using multi-nutrient response regression with 15 treatments of NPK in three set experiments. Then, Li et al. (2023) found the ratio of NPK 1.53:1.00:3.36 to obtain the total sugar content of melon fruit about 13.34% using 23 treatments by regression surface response method. This study focuses on conducted NPK doses to produce excellent levels of sugar content (≥16%) and uses a simple regression method to simplify the methodology.

Materials and Methods

This research was conducted from August until November 2023 at a greenhouse in Cikampek, Karawang, West Java. The tools used include TDS and pH meters, SPAD-502 Konika Minolta chlorophyll meters, GBC Savanta AA spectrophotometer, and other laboratory tools. Meanwhile, materials used in this experiment are 'Cantaloupe' SweetNet8 seeds, cocopeat, rice husk biochar, raw water (TDS<100 ppm), ropes, polybags, sample plastic, Luff Schoorl reagent, and other laboratory materials.

This study's NPK fertilizer used PT Pupuk Kujang's formulas. NPK formulas should have highly different compositions for regression modeling purposes. Thus, NPK formulas are determined by the nutrition dominance principle to obtain different plant responses. Its were NPK 13-13-13 (generic), NPK 16-9-9 (N dominant), NPK 9-9-25 (K dominant), NPK 12-6-20 (NK dominant), and NPK 9-13-23 (PK dominant).

The experiment was a Completely Randomized Design (CRD) with five treatments and four replications. Each treatment used 40 plants on soilless culture integrated with a fertigation system. Nutrition solution was applied six times daily, about 2.000 mg/L in various volumes, followed by plant stages. Pollination is carried out manually using a paintbrush. Finally, fruit is harvested at 40 days after pollination.

The observation parameters were plant dry matter and leaf NPK uptake (BPSITP, 2023), leaf area (Toebo et al., 2019), fruit weight, and fruit sugar content (Lubis et al., 2022). Data were analyzed using Pearson correlation and a simple regression method using Minitab 21.4 software.

Results and Discussion

Observation was conducted on growth parameters, such as leaf area, leaf nutrient uptake (Table 1) and plant dry matter (Table 2) at 3 WAP and 7 WAP. Evaluation at 3 WAP to observe plant condition in the maximum vegetative phase and 7 WAP for the maximum generative phase (Zhang et al., 2016).

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Table 1. Leaf area and leaf nutrients uptake of melon in response to various NPK treatments.

Treatments	Leaf Area (cm^2)		Leaf Nutrients Uptake (%)					
	3 WAP	7 WAP	3 WAP			7 WAP		
			N	P_2O_5	K_2O	N	P_2O_5	K_2O
NPK 13-13-13	296.92	404.98	5.12	0.73	1.88	3.86	1.05	1.88
NPK 16-9-9	246.33	352.58	5.29	0.89	1.57	4.41	1.01	1.98
NPK 9-9-25	268.50	393.89	4.53	0.63	1.88	3.31	0.44	3.43
NPK 12-6-20	264.58	409.69	4.76	0.85	2.03	3.94	0.71	4.20
NPK 9-13-23	320.33	403.21	4.55	0.87	2.07	3.69	0.71	3.87

Note: WAP = week after planting. N = nitrogen. P_2O_5 = phosphate. K_2O = potassium.

Table 2. Dry weight, fruit weight, and fruit sugar content in response to various NPK treatments.

Treatments	Dry Weight (g)		Fruit Weight Average (Kg)	Fruit Sugar Content (%)		
	3 WAP	7 WAP		Reducing	Sucrose	Total
NPK 13-13-13	31.00	167.13	1.26a	3.34	7.05	10.39
NPK 16-9-9	31.98	170.83	1.21a	3.36	7.10	10.46
NPK 9-9-25	35.73	166.63	1.30a	3.38	9.22	12.60
NPK 12-6-20	30.68	184.50	1.30a	3.41	9.28	12.69
NPK 9-13-23	29.93	218.90	1.26a	3.44	9.36	12.79

Note: WAP = week after planting.

Table 3. Pearson correlation coefficient between total sugar content and other parameters.

Parameter	Total Sugar (%)	
	Pearson Correlation (95%)	P-Value
Reducing Sugar (%)	0.863	0.060
Sucrose Sugar (%)	1.000	0.000*
N dose (g/plant)	-0.829	0.083
P_2O_5 dose (g/plant)	-0.285	0.642
K_2O dose (g/plant)	0.932	0.021*
N uptake 3 WAP (%)	-0.943	0.016*
P_2O_5 uptake 3 WAP (%)	-0.081	0.897
K_2O uptake 3 WAP (%)	0.755	0.140
N uptake 7 WAP (%)	-0.640	0.245
P_2O_5 uptake 7 WAP (%)	-0.877	0.051
K_2O uptake 7 WAP (%)	0.973	0.005*
Leaf area 3 WAP (cm^2)	0.265	0.667
Leaf area 7 WAP (cm^2)	0.545	0.342
Dry weight 3 WAP (g)	0.103	0.870
Dry weight 7 WAP (g)	0.569	0.317
Fruit weight average (Kg)	0.757	0.139

Note: *significant difference at the 0.05 probability level. Correlation 0.00-0.19 (very low); 0.20-0.39 (low); 0.40-0.59 (moderate); 0.60-0.79 (high); 0.80-1.00 (very high). Antagonism correlation (-); simultaneous correlation (+). WAP = week after planting. N = nitrogen. P_2O_5 = phosphate. K_2O = potassium.

Data were analyzed using Pearson correlation analysis to determine parameters that correlated with total sugar content as the main target of this study. Following Table 3, the total sugar content was only positive and significantly correlated with sucrose sugar, K_2O dose, and K_2O uptake at 7 WAP. However, total sugar negatively correlates with N uptake at 3 WAP.

The negative correlation (-0.943) means that the N uptake at 3 WAP has no linear response with total sugar content on melon fruit. Nitrogen has a dominant role in the vegetative phase of plants. Kumar et al. (2021) said that nitrogen on melon plants can increase melon fruit's dry matter and flesh color. A nitrogen deficiency in melon plants affects the fruit's decreasing weight and size (Grasso et al., 2022). So, if the N uptake at 3 WAP increases, the total sugar content will decrease.

Table 4. NPK fertilizers doses by multiple response prediction.

Categories*	Total Sugar Target (%)	Doses (g/plant)		
		N	P_2O_5	K_2O
Poor	8	31.56	23.99	35.26
Average	12	31.56	23.99	42.84
Good	14	31.56	23.99	46.63
Excellent	16	31.56	23.99	50.42

Note: *Total sugar categories following to Adams (2023).

The results of the correlation analysis in Table 3. showed a very high significance (0.000 p-value) and positive correlation (1.000) between sucrose sugar content and total sugar content. According to Stein & Granot (2018), sucrose is the final sugar product of the photosynthesis process, and it is distributed by the phloem vessels to all parts of the plant. Chaudhry and Varacallo (2023) stated that plants will store carbohydrates in simpler forms, such as sucrose, as food reserves so that when needed, the sucrose will be hydrolyzed into glucose through the glycolysis process.

Table 3. also describes essential information about the correlation between the total sugar content and potassium parameters, such as K_2O dose and K_2O uptake at 7 WAP were 0.932 and 0.973, respectively. It indicated a very high correlation. Potassium has a vital role in several plant physiological functions, such as carbohydrate metabolism, enzyme activity, osmotic regulation, water use efficiency, nitrogen element absorption, protein synthesis, and assimilate translocation (Dreyer et al., 2017; Rangel et al., 2018; Ho et al., 2020; Mostofa et al., 2022; Wang et al., 2023). Furthermore, Wang et al. (2024) explained that potassium is also crucial in the sugar synthesis process in leaves because it can influence the expression of genes that regulate sugar metabolism and assimilate transport. Potassium deficiency causes reduced sugar translocation from source to sink tissue (Koch et al., 2019; Ho et al., 2020). Moreira et al. (2022) stated that potassium deficiency can reduce sugar levels in melon fruit.

According to the results in Table 3, potassium has a high positive significant correlation to total sugar content in melon fruit. That result is similar to Table 4, which determines NPK doses by multiple response prediction. In Table 4., the recommendation of N and P_2O_5 to produce total sugar content in each category were similar to 31.56 g/plant and 23.99 g/plant, respectively, except for the doses of K_2O. This situation means that the K2O dose influences the total sugar content.

Conclusion

The total sugar content is the main target in the melon fruit production. It has a strong and significantly positive correlation with potassium in NPK fertilizer treatments, such as parameters of K_2O dose and K_2O uptake at 7 WAP were 0.932 and 0.973, respectively. NPK formula fertilizer containing 31.56 g N, 23.99 g P_2O_5, and 50.42 g K_2O can be used by the grower to produce melon fruit with excellent fruit sugar content according to regression model $y = -50.7 + 1.079\ N + 0.251\ P_2O_5 + 0.528\ K_2O$. In the following research topic, the recommendation of NPK doses should be tested to ensure the actual effect on the melon plant.

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Bioremediation of Pb and Cd contaminated soil by mycorrhiza and biochar treatment and its effect on growth and yield of shallot

Abstract. The contamination of shallots in the food chain by heavy metals such as lead (Pb) and cadmium (Cd) is caused by chemical fertilizers and pesticides. The study aimed to determine the growth and yields of shallot cultivated on soil contaminated with Pb and Cd using mycorrhiza and biochar. The study was conducted in the screen house at Jenderal Soedirman University, Faculty of Agriculture, from April to September 2020, and it was carried out using a factorial Randomized Completely Block Design that involved three replications and two factors. The first factor of mycorrhiza dosage comprised 0, 1, and 2 g/pot, and the second factor of biochar dosage comprised 0, 2.5, 5, and 10 t/ha. The plant height, leaf area, growth rate, number of leaves, total root length, net assimilation rate, leaf chlorophyll, the percentage of root infection, P uptake by plant tissue, tuber weight, harvest index, the effectiveness of absorption and removal of heavy metals were the variables recorded. The results showed that applying biochar at 2.5, 5, and 10 t/ha and mycorrhiza at 1 and 2 g/pot could increase plant height and the percentage of root infection. The application of mycorrhiza at 1 and 2 g/pot increased P uptake by plant tissue.

Keywords: Biochar · Bioremediation · Heavy metal · Mycorrhiza · Shallot

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Introduction

Shallot (*Allium ascalonicum* L.) is a horticultural commodity with high economic value. Furthermore, it is used as a cooking spice and raw material for medicine and the food industry (Saptana et al., 2021). Shallots contain Fe, antioxidants, antifungal, and anticancer properties (Abdelrahman et al., 2017). Therefore, a key challenge in vegetable production and marketing is raising public knowledge of the importance of food safety and quality. Vegetable cultivation, particularly of shallots, heavily relies on agrochemical inputs, often resulting in varying crop quality and potential heavy metal contamination in the bulbs. Furthermore, the main contributor to contaminated food is the buildup of heavy metals in soil, water, or plants. Rapid industrial development and excessive use of pesticides and fertilizers have increased heavy metal deposition in the environment. (García-Díaz et al., 2017; Zarcinas et al., 2004). These events have raised a global concern about soil pollution. Plants produce a substance known as thiols, which has a low molecular weight and thus, has a high affinity for toxic metals. Metal ions would signal to plants to produce thiols as a defense mechanism against heavy metal stress by accumulating heavy metals and synthesizing low molecular weight metal-binding proteins (Borisova et al., 2016). The plant root system is suitable for absorbing heavy metals such as Pb and Cd when found in the soil (Ali et al., 2019).

Contamination of soil with Cd and Pb can have negative effects on soil quality, plant growth, and soil microorganisms. Effects of Cd and Pb on soil properties include Decreased metabolic activity of bacteria and other soil microorganisms, disrupted plant development, Reduced fertility and plant nutrient uptake Soil pollution (Esringü et al., 2021; Rolka & Wyszkowski, 2021). The effect of potentially toxic elements can reduce soil microbial activity, soil microbial community composition, soil enzyme activity, and soil physiochemical properties thereby reducing soil fertility (Bansal, 2018).

Lead (Pb) accumulates in plant parts in the form of leaves, stems, and roots. Lead absorption by roots occurs when water-insoluble compounds convert into water-soluble forms, allowing lead to bind with root mucus carboxyl groups, which limits the root's protective heavy metal bonds. The absorption of lead in the leaves is through a passive absorption process in the stomata of the leaves, which causes the amount of lead levels to be greater due to absorption from the air. If the plant is consumed by humans, it can cause lead poisoning in the form of nausea, vomiting, severe abdominal pain, brain dysfunction, severe anemia, and kidney damage (Almaroai & Eissa, 2020). The threshold value for lead is 1.0 ppm in soil and 0.2 ppm in plants, while cadmium in shallots ranges from 0.135 to 0.285 ppm, exceeding the threshold limit of 0.1 ppm.(Nadir et al., 2018).

Cadmium (Cd) generally comes from factory waste and household domestic waste. If plants contaminated with cadmium are consumed by humans, it can cause lung obstruction, slow growth, osteoporosis, and disturbances in the balance of calcium and phosphate content in the kidneys which results in a deficiency of B vitamins resulting in softening of the bones (Yusuf et al., 2016). The threshold value for cadmium in soil is 0.24 ppm, while the threshold for cadmium in plants is 0.1 ppm (Nadir et al., 2018).

However, a solution is needed to overcome and rehabilitate soils contaminated with heavy metals, such as the use of an amendment substance called biochar. Biochar is produced by the pyrolysis of waste from different sources, such as forests or agriculture (Hussain et al., 2017) and it is used as an alternative for restoring and remediating soil contaminated with synthetic fertilizers and pesticides. The absorption mechanism of heavy metals Pb and Cd by biochar is dominated by complexation with functional groups, cation exchange, and precipitation. The hydrogen atom in the –COOH carboxyl group can be released as an H^+ ion or undergo deprotonation, so that it has the opportunity to form complexes with metal ions such as lead and cadmium (Cui et al., 2020). Furthermore, biochar also binds to some elements such as N, Ca, and K (Puga et al., 2015).

The use of biofertilizers is an alternative way of suppressing synthetic fertilizer usage and restoring microorganisms in the soil. An example of biological fertilizer is the Vesicular-arbuscular mycorrhizal fungi which is known to boost the uptake of P supplements through its roots by expanding the absorption system of the hyphae which extends into the soil to absorb P ions from the soil minerals or other organisms, and translocate them to the roots of the host plant (Susanti et al., 2018). Arbuscular mycorrhizal fungi function to retain these heavy metals so they are not absorbed into plant tissues, or reduce heavy metal levels so that plants are safe for consumption (Nadir et al., 2018).

Based on the previous studies and literature on the role of mycorrhiza and biochar in the process of heavy metal abandonment, this research is interesting to carry out. This study aims to determine the role of biochar and mycorrhiza on the yield of shallots and its effect on Pb and Cd remediation.

Materials and Methods

Preparation of media. The experimental pot was prepared in April - August 2020 at the Screen House, in Jenderal Soedirman University, Faculty of Agriculture, Purwokerto, Karangwangkal (110 masl). Alluvial soil was the medium used for the experiment and it was obtained from the Brebes area at an initial pH of 7.27. Heavy metals such as Pb and Cd were present in the soil at a concentration of 29.05 ppm and 3.66 ppm. Furthermore, the media obtained from the soil was dried to remove moisture and filtered using a filter of size 2 mm and weighed to obtain 6 kg per pot.

Research design. Randomized factorial complete block design (RCBD) was used for this study and it consisted of 2 factors. The first was mycorrhizal which was made up of 3 levels while the second, was Biochar which comprised 4 levels. To achieve 36 experimental units, 12 treatments were combined and repeated thrice. The experimental unit was made up of 4 plants which were used to obtain 144 pots.

Biochar application. Corn cob, as the main material in this study, was collected from maize fields in Jakenan Village, Jakenan District, Pati Regency, Central Java Province, Indonesia. Corn cob biochar is produced by the Center for Agricultural and Environmental Research. Furthermore, the Biochar was applied to the growth medium and left for seven days before planting was conducted. A temperature range of 100-300°C was utilized for 3-5 hours, followed by the removal of the combustion temperature after the pyrolysis process. The material was thereafter allowed to cool for 12 hours. Then, mash until granule 0.2 mesh is ready for application (Ratnasari et al., 2020). The chemical analysis of corn cob biochar shows it contains 15.9% fixed carbon, 9.2% ash, and has a C/N ratio of 4.39. It consists of 76% carbon (C), 3.2% hydrogen (H), 3% nitrogen (N), and 17.8% oxygen (O).

Mycorrhiza application. The mycorrhiza used in this study was *Glomus* sp type, which is explored from corn fields. Then propagation is carried out and coated with a carrier material in the form of zeolite 1 g of mycorrhiza biofertilizer, containing 7 spores of *Glomus* sp, was added to the growth medium and left for 7 days before planting the tuber of shallot.

Plant cultivation and crop management. The used shallot seeds were the Bima Brebes variety. Two seedlings coated with fungicide were planted in the pot, with 2/3 of the tuber deeply inserted into the soil. 0.6 grams per pot of urea fertilizer was used to fertilize the soil. The fertilizers were added twice after planting (Day 7 and Day 20). 500 ml of water was used for watering every 2 days, while weeding was carried out every 10 days. The plant was harvested when it attained a 65 DAP, the leaves began to turn yellow and wilt, and the tubers started sticking out to the surface of the planting medium.

Variable observed. The variable determined was the number of leaves, leaf area, total root length, plant growth rate, net assimilation rate, chlorophyll content, P uptake, number of bulbs, weight of bulb, harvest index, root infection of mycorrhizae, Pb and Cd in tissue plant of shallot, effectiveness of absorption and removal of Pb and Cd.

The net assimilation rate. This variable was measured based on the dry weight and leaf area of the plant per unit time with the following formula (Shon et al., 1997):

$$NAR = \frac{1}{A} \times \frac{\Delta W}{\Delta t}$$
$$= \frac{(\text{Log A2-Log A1})}{(\text{A2-A1})} \times \frac{(\text{Log W2-Log W1})}{(\text{t2-t1})}$$

NAR = Net Assimilation Ratio
A1 = leaf area on measurement time 1
A2 = leaf area on measurement time 2
W1 = plant biomass on measurement time 1
W2 = plant biomass on measurement time 1
t1 = measurement time 1
t2 = measurement time 2

Leaf chlorophyll content (mg/L). Its variable is determined by the following formula (Dharmadewi, 2020):

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Chlorophyll a = 1.07 (OD 663) – 0.094 (OD 644)
Chlorophyll b = 1.77 (OD 644) – 0.28 (OD 663)
Total Chlorophyll = 0.79 (OD 663) + 1.076 (OD 644)
Remark:
OD = Optical Density of spectrophotometer.

P uptake. The calculation of P uptake is determined by the following formula (Eviati & Sulaeman, 2009):

P content (%) = ppm curve x ml extract/1,000 ml x 100/mg sample x B.A. P/B.M. PO4 x fp x fk
= ppm x curve 50/1,000 x 100/250 x 31/95 x fp x fk
= ppm x curve 0.02 x 31/95 x fk

Remark:
ppm curve = sample rate obtained from the relationship curve between series rates standard with its reading after being corrected by the blank.
100 = conversion factor to %
1,000 = conversion factor to ppm (mg kg-1)
fp = dilution factor (10)
fk = moisture content correction factor = 100/(100 - % moisture content)
Percentage = % P in plants
P absorption = Dry weight of the plant x P content of the plant

Root infection. The measurement of the percentage of root infections uses the following formula (Adetya et al. (2019):

$$(\%)\ \text{Infection} = \frac{\text{(Number of infected roots)}}{\text{(Total number of roots)}} \times 100\%$$

Removal efficiency. The effective absorption rate of heavy metals is also known by the Environmental Protection Agency EPA. It refers to the ability of plants to absorb heavy metals. The EPA was obtained with the formula below (Herliana et al., 2021):

$$\text{RE}\ (\%) = \frac{\text{IMC-FMC}}{\text{FMC}}\ / \times 100\%$$

Remark:
RE = removal efficiency
IMC = initial metal concentration in soil
FMC = final metal concentration in soil

Data analysis. All data were evaluated using analysis of variance (ANOVA). The effectiveness of treatment was obtained using a 95% confidence level. The variance obtained was significantly different. Therefore, it was compared with treatments using a follow-up test called Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Results and Discussion

Plants have been able to adapt to heavy metal stress, however, the addition of biochar and mycorrhizal brought a change in the growth, yield, and removal of this heavy metal (Table 1).

Table 1. Effect of mycorrhiza and biochar application on shallot growth and yield.

Treatment	Variables								
	NL (g)	LA (cm^2)	TRL (cm)	PGR (g/day)	NAR ($g/cm^2/day$)	CC (mg/l)	P uptake (ppm)	NB	WB (g)
Mycorrhiza									
0 g/pot	26.33	47.24	295.40	0.096	0.048	13.12	132.48 b	7.048 b	2.53
1 g/pot	24.62	52.52	292.52	0.085	0.044	11.84	167.68 a	8.181 a	2.67
2 g/pot	25.84	68.66	335.65	0.129	0.018	12.80	177.25 a	8.362 a	2.75
Biochar									
0 t/ha	20.93 b	52.32	307.29	0.071	0.040	12.70	139.00	6.444 c	2.19a
2.5 t/ha	25.73 a	59.53	296.76	0.106	0.020	12.50	179.27	7.463bc	2.60b
5 t/ha	28.67 a	62.78	293.34	0.145	0.029	12.66	155.47	7.806bc	2.81bc
10 t/ha	26.60 a	65.25	334.04	0.090	0.058	12.48	162.80	9.741a	3.02c
CV (%)	17.23	8.25	27.19	25.40	21.32	14.42	22.07	21.46	24.55

Remarks: NL: Number of leaves, LA: Leaf Area, TRL: Total root length, PGR: Plant Growth Rate, NAR: Net Assimilation Rate, CC: Chlorophyll content, P Uptake, NB: Number of Bulbs, WB: Weight of Bulb. The number followed by the lowercase letter shows a significant difference based on the DMRT test with p=0.05

Effect of mycorrhiza on growth and yield of shallots on heavy metal contaminated soil. The mycorrhiza used in this experiment was from the *Glomus* species. While it did not affect growth variables, it significantly increased the number of bulbs by enhancing nutrient, water, and mineral absorption through its root-associated hyphae, thereby optimizing bulb formation. The addition of 1 g mycorrhiza/pot or 2 g mycorrhiza/pot was able to increase P uptake in the tissue plant. When 1 and 2 g/pot of mycorrhizal were applied, the P absorption of plant tissue was 167.68 ppm and 177.25 ppm, respectively. However, there is no significant difference between the two values. However, there was a significant difference in the treatment when mycorrhiza was not applied, where the P uptake was 132.48 ppm (Table 1). Mycorrhiza is a beneficial fungus that forms symbiotic associations with many plants. They enhance the uptake of nutrients and water through increased root surface area and a wide hyphal system. Furthermore, the efficiency of AMF inoculation is regulated by several factors such as the type of mycorrhizal and plant, availability of nutrients for crops, climate, and stress factors (Chen et al., 2018). Mycorrhiza symbiosis with almost all plant roots contributes significantly to the availability of plant nutrients, especially to phosphorus absorption (Smith et al., 2003). First, due to its enormous surface area, the hyphal tissue of Arbuscular Mycorrhiza Fungi (AMF) is very efficient in nutrient absorption (Plenchette et al., 2005). Fungal partners form extensive extraradical mycelium in the soil, enhancing the root's absorption area (Lioussanne et al., 2009).

Furthermore, from this study, AMF inoculation was known to increase P uptake significantly. The role of mycorrhizal fungi in the acquisition of mineral nutrients, especially phosphorus was explained previously by Bolduc & Hijri, (2010) who demonstrated the increase in growth of *Allium cepa* as a result of inoculation with mycorrhiza fungi (Citterio et al., 2005). Mycorrhizal fungi improve plant growth by enhancing nutrient uptake under phosphorus-limiting conditions. (Bano & Ashfaq, 2013) also stated that the growth of the mycorrhizal inoculated plant has a positive relationship with the growth of the host plant, thereby, increasing nutrient uptake through an external mycelium by expanding the root absorption surface or by producing a chemical compound that causes the release of nutrient.

Effect of biochar on the growth and yields of shallots on soil contaminated with Pb and Cd. Biochar has a significant effect on the number of leaves, bulbs, and weight. Furthermore, it was seen that soil without biochar gave a low yield of 20.926 cm, which is significantly different from soil with biochar treatment of 2.5, 5, and 10 t ha, which gave a yield of 25.731, 28.667, and 26.602 cm respectively. In terms of bulb variables, soil without biochar gave a low yield of 6.444 but this was not significantly different from biochar treatment of 2.5 and 5 t/ha, which gave a yield of 7.463 and 7.806 number of bulbs per pot. The highest yield of 9.741 bulbs was produced by 10 t/ha of biochar.

The existence of biochar in various agricultural waste materials confirms the presence of different minerals such as phosphorus and nitrogen. These minerals increase soil productivity leading to a high yield of crops. Therefore, they are vital for plant growth and for boosting soil productivity. Nurida (2014) stated that the addition of biochar to the soil increases the N and P minerals present. Furthermore, it is known to have a high water-holding capacity which helps prevent nitrogen minerals from being washed away easily, making it more available to plants. Sohi, et al. (2010) found that the utilization of biological fertilizer and biochar had a notable impact on the vertical growth of the *Brahiaria decumben* grass species. This growth is affected by the rate of nitrogen uptake by the soil which increases after biochar application. According to Song, et al. (2018), adding biochar to land improves the available P and N-total. Satriawan & Handayanto (2015) explained that plants require phosphorus (P) nutrients for development, root and seed formation, faster flowering, and maturation. As a result, the amount of P element present in the soil determines the P nutrients needed by plant roots.

Effect of mycorrhiza to Cd and Pb on tissue plant and removal efficiency. Figure 1 shows that the utilization of mycorrhiza significantly affects the absorption of Pb and Cd. In soil that was not inoculated, the uptake of Pb by plant tissue gave a high yield of 16.65 ppm, which is significantly different from the soil with 1 and 2 g mycorrhizae/pot, where the Pb uptake was 13.228 ppm and 11.269 ppm, respectively. The uptake of Cd gave a high yield of 1.433 ppm when no inoculation was conducted. However, this result was significantly different from the soil with 1 and 2 g mycorrhiza/pot, where the Cd uptake was 1.203 ppm and 1.07 ppm, respectively.

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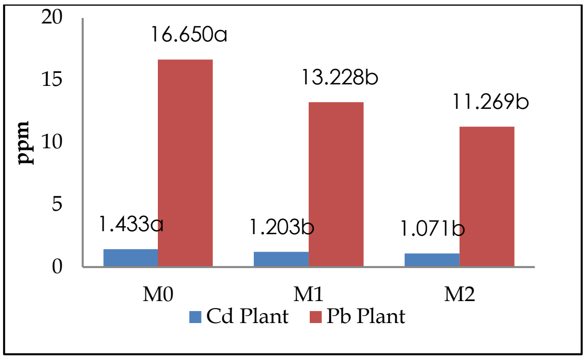

Figure 1. Effect of mycorrhiza application to Cd and Pb on plant uptake.
Notes: (*) M0 = 0 g mycorrhiza/pot; M1 = 1 g mycorrhiza/pot, M2 = 2 g mycorrhiza/pot

The application of mycorrhiza to the soil is very significant in the removal of heavy metal effectively as shown in Figure 2. Therefore, planting medium without mycorrhizae removes Pb at a small percentage of 33.74%. This value is significantly different from media inoculated with 1 and 2 g mycorrhizae/pot which remove Pb at 53.94% and 65.26%. Furthermore, the percentage of Cd removal in the media that was not inoculated with mycorrhizae was 29.74%. This result is significantly different from media inoculated with 1 and 2 g mycorrhiza/pot which remove Cd at 48.33% and 55.62%. The removal ability of heavy metals was highly classified refers to the EPA standard.

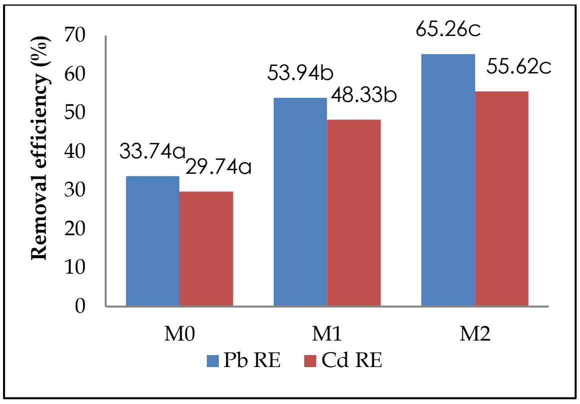

Figure 2. Effect of mycorrhiza application to Cd and Pb on Removal Efficiency (RE).
Notes: (*) M0 = 0 g mycorrhiza/pot; M1 = 1 g mycorrhiza/pot, M2 = 2 g mycorrhiza/pot

Mycorrhizae have the potential to aid in the bioremediation of heavy metal contamination in soil. Arbuscular mycorrhizal fungi reduce the threat of heavy metals by secreting several compounds, which affect the deposition of metals in polyphosphate granules in the soil, adsorb metals to the fungal cell walls, and chelate heavy metals in the fungi. Organic acids and glomalin released from plants and fungi also play an important role in immobilizing heavy metals in the soil. Plant-colonized arbuscular mycorrhizal fungi release organic acids, which increase the sequestration and absorption of heavy metals, and organic acids are precipitated as chelated polyphosphate granules and heavy metals that are immobile in the soil. Soil management applications reduce the ability of mycorrhizal sporulation and colonization by disrupting the extra-radical mycelium network. Disturbance of the hyphal tissue reduces its surface area. To prevent stressful conditions in the environment, AMF grows a wider mycelium (Herath et al., 2021). The bioaccumulation and immobilization of hazardous heavy metals in soil is also facilitated by the endomycorrhizal relationship. Mycorrhiza is a symbiotic relationship between fungus and higher plant roots. Fungus infiltrates plant roots and produces hyphae, arbuscules, and vesicles. As a result of this interaction, nutrients, particularly phosphorus, are transported. Cadmium and lead, for example, interfere with several physiological and biochemical processes in plants, including photosynthesis, respiration, nitrogen, and protein metabolism.

Effect of biochar to Cd and Pb on tissue plant and removal efficiency. Biochar can decrease heavy metal uptake in plant tissue (Figure 3). Application of biochar 10 t/ha and 7.5 t/ha showed a significant effect on Pb in plant tissue were 9.849 ppm and 11.982 ppm lower than biochar 2.5 t/ha treatment and without biochar application with the value of 15.680 ppm and 16.684 ppm, respectively. Biochar application has not been able to significantly reduce Cd uptake, but its value tends to decrease.

Biochar also can remove heavy metals (Figure 4), the use of biochar has a significant effect on the effective removal of Pb and Cd. Pots that lack biochar removed Pb at 36.91%, which is low. This is significantly different from pots that contain biochar 2.5 and 5 t/ha, which removed Pb at 41.329% and 46.065%. The highest percent is seen when 10 t/ha biochar is applied to a pot. However, the application of 2.5 t/ha biochar was able to remove Cd at 36.82%, which is not significantly different from the 5 and 10 t/ha biochar treatments, which remove Cd at 42.54% and 49.73%, respectively.

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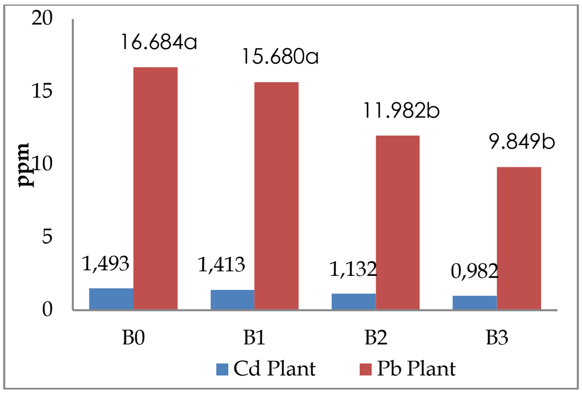

Figure 3. Effect of Biochar Application to Cd and Pb on Plant Uptake.
Notes: (*) B0 = 0 t/ha biochar, B1 = 2.5 t/ha biochar, B2 = 5 t/ha biochar, B3 = 10 t/ha biochar

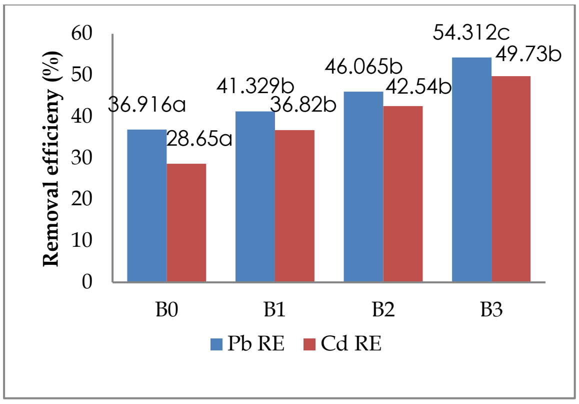

Figure 4. Effect of Biochar Application to Cd and Pb on Removal Efficiency (RE).
Notes: (*) B0 = 0 t/ha biochar, B1 = 2.5 t/ha biochar, B2 = 5 t/ha biochar, B3 = 10 t/ha biochar

The corn cob biochar used in the present study contained 69.937% cellulose, 17.797% hemicellulose, and 9.006% lignin. If the elements in corn cobs are used as biochar, they will be deformed which can increase the availability of nutrients and the amount of c-organic in the soil. Increasing C-organic indirectly provides a good habitat for soil microbes, which play a role in decomposing organic matter in the soil to increase nutrient availability and bind heavy metals that are harmful to soil and plants (Rodriguez et al., 2019).

Biochar can stabilize heavy metals in polluted soil, absorb heavy metals, and improve the physical, chemical, and biological qualities of the soil. The application of biochar can reduce the mobility of polluted heavy metals in the soil so that heavy metals in the form of radicals will not be absorbed by plants (Yao et al., 2011). Biochar can reduce the mobility of lead and cadmium radicals in soils with low pH and non-electrostatic absorption. Biochar can eliminate the activity of heavy metal ions in contaminated soil so that heavy metals do not enter the food chain system in living things because they will be immediately leached and precipitated (Tan et al., 2015).

The application of biochar resulted in a total lead concentration in shallot plantations of 17.53-22.59 mg/kg (Dewi et al., 2022). This concentration is far below the critical limit of heavy metals needed for agricultural land, namely 100-400 mg/kg of lead. This is because biochar can reduce lead in roots, tubers, and leaves. Total soil cadmium concentrations in shallot plantings that were applied with biochar were between 1.01-1.46 mg/kg, and this value was still below the critical limit of 3-8 mg/kg. The addition of biochar can change the bioavailability and mobility of cadmium in the soil. These steps are connected to many processes, including redox reactions, precipitation, adsorption, and complexation.

Interaction of mycorrhiza and biochar factors affect root infection and plant height. There was a significant effect of interaction between biochar and AMF factor on the root infection (Figure 5) and plant height (Figure 6). The 81.7% root infection was recorded when 2 g mycorrhizae/pot were combined with 10 t/ha of biochar. Treatment of 1 g mycorrhiza/pot combined with 10 t/ha of biochar was shown to increase plant height by 38.06 cm. However, this has no significant difference with mycorrhizae 2 g/pot combined with biochar 1 t/ha which increases plant height by 37.89 cm.

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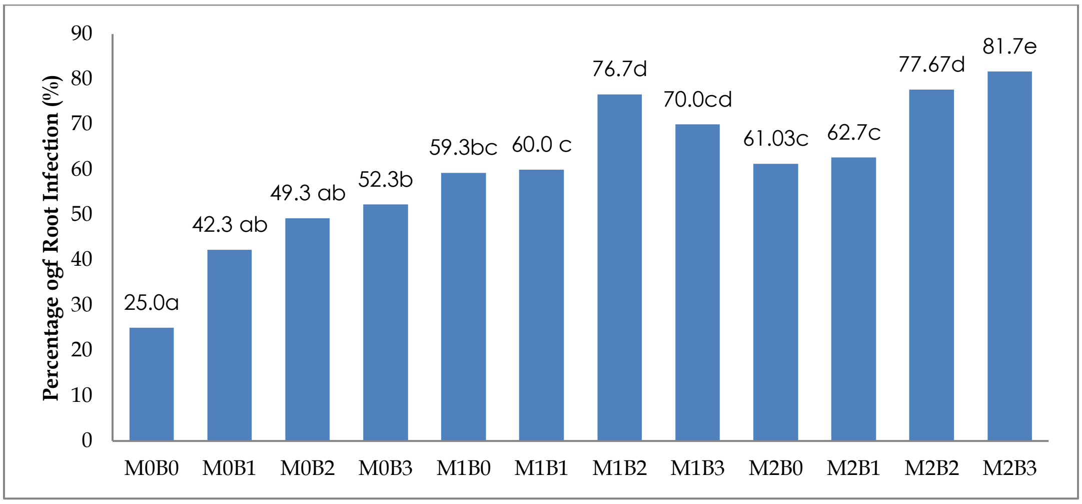

Figure 5. Interaction of Mycorrhiza and Biochar Factors Affect Root Infection.

Note: (*) M0B0 = no treatment M0B1 = 0 mycorrhiza and 2.5 t/ha biochar M0B2 = 0 mycorrhiza and 5 t/ha biochar M0B3 = 0 mycorrhiza and 10 t/ha biochar M1B0 = 1 g mycorrhiza/pot and 0 t/ha biochar M1B1=1 g mycorrhiza/pot and 2.5 t/ha biochar M1B2=1 g mycorrhiza/pot and 5 t/ha biochar M1B3=1 g mycorrhiza/pot and 10 t/ha biochar M2B0 = 2 g mycorrhiza/pot and 0 t/ha biochar M2B1= 2 g mycorrhiza/pot and 2.5 t/ha biochar M2B2= 2 g mycorrhiza/pot and 5 t/ha biochar M2B3= g mycorrhiza/pot and 10 t/ha biochar

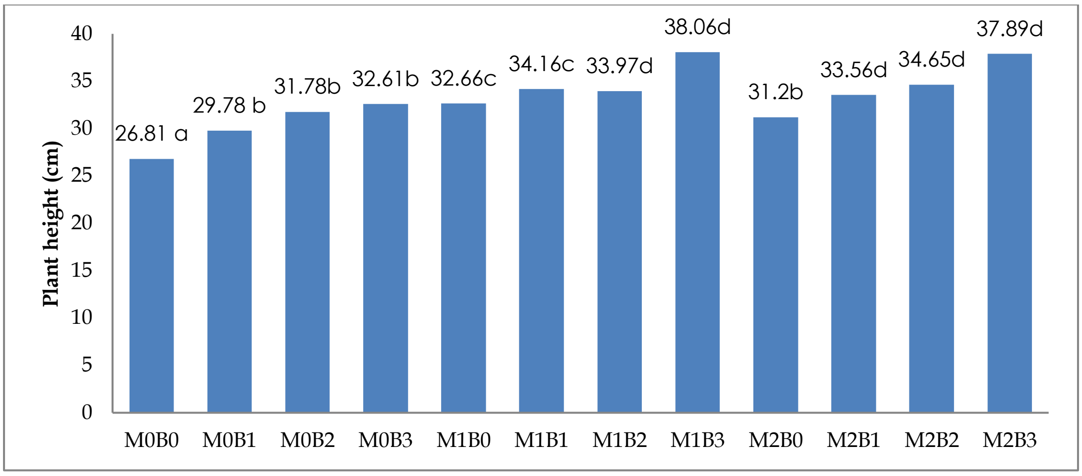

Figure 6. Interaction of Mycorrhiza and Biochar Factors Affect Plant Height.

Note: M0B0 = no treatment M0B1 = 0 mycorrhiza and 2.5 t/ha biochar M0B2 = 0 mycorrhiza and 5 t/ha biochar M0B3 = 0 mycorrhiza and 10 t/ha biochar M1B0 = 1 g mycorrhiza/pot and 0 t/ha biochar M1B1=1 g mycorrhiza/pot and 2.5 t/ha biochar M1B2=1 g mycorrhiza/pot and 5 t/ha biochar M1B3=1 g mycorrhiza/pot and 10 t/ha biochar M2B0 = 2 g mycorrhiza/pot and 0 t/ha biochar M2B1= 2 g mycorrhiza/pot and 2.5 t/ha biochar M2B2= 2 g mycorrhiza/pot and 5 t/ha biochar M2B3= g mycorrhiza/pot and 10 t/ha biochar

The use of biochar has helped improve the soil physical and biochemical properties, which has increased plant production (Hossain et al., 2020). It carries out these functions by increasing the nutrients and water content present in the soil, which aids in plant growth. According to Almaroai & Eissa, (2020), the nature of biochar includes the ability to retain and increase nutrients and water content, enhancement of soil biological, physical, and chemical properties, and improvement of soil structure and productivity. Mycorrhizal inoculation has a significant effect on plant height due to its role in metabolism, which occurs in plant roots, leading to an increased growth rate. Its metabolic activity is 2 to 4 times higher than that of non-mycorrhizal roots because of its ability to aid the absorption of mineral salts by increasing the supply of hydrogen ions (Liu et al., 2021).

In addition, the application of biochar in mycorrhizal treatment has been found to have the potential to enhance the rate of root infection. However, the observed increase in root infection percentage did not reach statistical significance, as it ranged from 59.30 to 84.30%. (Yang et al., 2023) state that an increase in mycorrhizal doses in roots tends to increase the root infection. Mycorrhizae promotes plant growth, development, stress tolerance, soil remediation, carbon sequestration, food safety, and agricultural sustainability

Soil given biochar consistently has an increased C content which is more stable than soil without biochar. Its high surface area and porosity made it possible for plants to absorb or retain nutrients, water, and also act as a habitat for growth of beneficial microorganisms. Biochar is suitable for improving the fertility of both chemicals, physical and biological soil (Jeffries, et al. 2003). Since AMF is reported to enhance N uptake, largely due to improved phosphate availability (Smith et al., 2011; Sohi et al., 2010), biochar can also supports mycorrhizal growth by providing a favorable habitat for soil microbes, aiding nutrient breakdown and plant absorption. So that biochar and mycorrhiza synergize with each other in efforts to improve soil.

Conclusion

Applying 2.5, 5, and 10 t/ha biochar are suitable for increasing plant height and the percentage of root infection. Application of mycorrhiza increases the P uptake and the number of the bulb by 25.26%. It also decreases the Pb and Cd uptake by 32.31% and 25.08%, respectively, leading to an increase in heavy metal removal.

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Growth and yield of bok choy (*Brassica rapa* L.) plants in post-rock excavation soils provided with phosphate-solubilizing bacteria and manure

Abstract. Elevated sand and gravel mining to meet market needs causes negative impacts on the environment, soil, and biodiversity. The government issued a regulation that requires revegetation as reclamation of mineral and coal mining. The conversion needs to overcome the physical and chemical characteristics of post-rock excavation soil that can be utilized by economic value plants such as bok choy. This experiment aimed to determine the growth and yield of bok choy on post-rock excavation soil by providing phosphate solubilizing bacteria (PSB) and various manure types. The study was conducted in Margasari Village, Buah Batu District, Bandung at an altitude of 671 m above sea level and at the Soil Biotechnology Laboratory, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung from April to June 2023. The experimental design used was a factorial Randomized Block Design (RBD) with two factors and repeated three times. The first factor was PSB isolate dose (without PSB; 5 mL polybag^{-1}; 10 mL polybag^{-1}; 15 mL polybag^{-1}, and 20 mL polybag^{-1}). The second factor was manure variety (cow, goat, and laying hen manure, each of 15 t ha^{-1}). The parameters observed comprised the soil and plant parameters. The results showed that there was no interaction between the dose of PSB isolate and various types of manure on the growth and yield of bok choy plants. The PSB isolate and manure had not been able yet to increase the growth and yield of Bok Choy (*Brassica rapa* L.) plants on post-rock excavation soil, mainly due to the soil nature.

Keywords: Bok choy · Growth · Manure · Phosphate Solubilizing Bacteria (PSB) · Post-rock excavation soil

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Introduction

High mining of sand and gravel to meet market needs causes various negative impacts such as air pollution, changing the pH of water to acid, as well as loss of biodiversity(Lei et al., 2016), decreasing soil quality in the form of a reduction in the ability to absorb and retain water, lack of nutrients, and susceptible to erosion(Ginting et al., 2018). On the other hand, the total P-nutrient content in the soil after rock excavation is found in high amounts of 156.44 mg.100g^{-1} in Cibeureum village, Sumedang district. Ramadhan et al., (2015), 135.69 mg 100g^{-1} in Cimareme, West Bandung district (Hidayat et al., 2020), 160.96 mg 100 g^{-1} originating from Mount Galunggung, Tasikmalaya district (Hidayat et al., 2023). This condition shows the potential for using soil from post-rock excavation for plant cultivation. However, technological input is needed to convert non-available P into available P that can be utilized by plants.

The utilization of post-rock excavation land is in line with regulations issued by the government as stated in Law No. 03 of 2020 concerning mineral and coal mining. This regulation requires revegetation as a criterion for successful reclamation. This indicates the potential for using post-mining soil as agricultural land. It is hoped that the conversion of land functions can be used to increase crop production, especially those with high economic value such as bok choy.

Bok choy (*Brassica rapa* L.) is a leaf vegetable that is widely consumed by the public. Nitrogen (N) potassium (K), and phosphorus (P) elements are needed in high amounts to support the growth of leaf vegetables(Bahri et al., 2020). The P element in leaf vegetables has an important role in photosynthesis, respiration, and other metabolic reactions (Bahri et al., 2020). Cultivating bok choy in post-rock excavation soil, which has minimal nutrients, means that efforts are needed to increase essential nutrients such as N and K, as well as releasing total P into available P, so that it can be utilized optimally by bok choy plants.

The efforts to overcome the problem of decreasing soil quality and low available P can be achieved by applying PSB. Soil pos-rock excavation has been identified to contain indigenous bacteria such as *Bacillus megaterium* and *Pseudomonas aeruginosa* (Sariwahyuni, 2012). PSB is a bacteria that can convert bound phosphate into soluble and is available through the production of organic acids such as 2-Ketogluconic and gluconic acid (Kishore et al., 2015). Furthermore, organic acids can chelate Fe, Al, and Ca ions bound to phosphorus and release them so that the P element becomes available and can be absorbed by the plants (Sharma et al., 2013; Campos et al., 2018) . Lovitna et al. (2021) showed that the application of PSB at a dose of 100% + 150% SP-36 on Alfisol soil could increase available P from 21.65 ppm (0 DAP) to 29.68 ppm (8 DAP). PSB performance can be optimized by adding organic material, especially in post-excavated rock soils that have very low C-organic content, such as C-organic in Cibeureum village, West Bandung district, 0.86% (Hidayat et al., 2020) and C-organic in Mount Galunggung Tasikmalaya district 0.19% (Hidayat et al., 2023).

Organic material can be plant or animal remains such as cow, goat, and chicken manure. These three animals are widely farmed in Indonesia so their manure is available in abundance and has the potential to be processed into fertilizer. According to Kadir et al. (2016) organic material can unite soil particles, increase the soil's capacity for water, and add nutrients N, P, K, Ca, Mg, S, and Fe. Apart from that, soil C-organic levels can also be increased by applying organic manure (Chairunnisya et al., 2017)

Organic materials can act as a source of food and energy for PSB microbes (Fitriatin et al., 2014). Meanwhile, PSB works to help decompose organic materials. The combined application of PSB and organic materials is expected to provide a synergistic effect on improving soil quality after rock excavation, so this research was conducted to determine the application of PSB and manure on the growth and yield of bok choy plants in post-rock excavation soil.

Materials and Methods

The research was conducted in Margasari Village, Buah Batu District, Bandung, West Java Indonesia at an altitude of 671 meters above sea level (6 º 57'11"S 107 º 38'56"E) and at the Soil Biotechnology Laboratory, Faculty of Science and Technology, University Islam Negeri Sunan Gunung Djati Bandung from April to June 2023.

The tools used in this research included a hoe, a 0.5 cm hole sieve, a ruler, digital scales, analytical scales, paper envelopes, plastic, polybags measuring 30 cm x 30 cm, watering can,

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stationery, thermohygrometer, seedling tray, plant labels, measuring cup, petri dish, micropipette, oven, autoclave, shaker, hot plate, Munsell Plant Tissue Color Book, LAF.

The materials used in this research include post-excavated soil from the mining area of Cimareme District, Bandung Regency. The organic materials have been applied 3 times during 3 growing seasons, green variety bok choy seeds, PSB was isolated from the exploration at the post-excavation site for Patrol rocks, Bandung Regency which is the collection of the Soil Biotechnology Laboratory, UIN Sunan Gunung Djati Bandung, cow manure, goat manure, laying hen manure, urea fertilizer, SP-36 fertilizer and KCl fertilizer, distilled water, mineral water, Pikovskaya broth media, Pikovskaya agar, and water.

The experimental design used was a factorial Randomized Block Design (RBD) with two factors, namely PSB isolate variety (m) with five treatment levels and manure variety (p) with 3 treatment levels, repeated 3 times. The first factor was the dose of PSB isolate, namely m_0: without PSB isolate, m_1: PSB isolate = 5 mL polybag^{-1}, m_2: PSB isolate = 10 mL polybag^{-1}, m_3: PSB isolate = 15 mL polybag^{-1}, m_4: PSB isolate = 20 mL polybag^{-1}. The second factor was the variety of manure, namely: p_1: cow manure 15 t ha^{-1} (30 g polybag $^{-1}$), p_2: goat manure 15 t ha^{-1} (30 g polybag^{-1}), p_3: laying hen manure 15 t ha^{-1} (30 g polybag^{-1}).

The observation parameters observed comprised on the soil and plant parameters. Initial soil analysis included soil texture, pH, C-Organic, C/N Ratio, N, P, K, and soil CEC (Cation exchange capacity). The manure's analysis included pH, C-Organic, N, P, K, and water content. Analysis of soil and manure for laying hens was carried out at the Laboratory of the Agricultural Instrument Standardization Agency (BSIP) for Vegetable Crops, Lembang, West Bandung Regency. Analysis of cow and goat manure content was at the Laboratory of the Department of Soil Science and Land Resources, Bogor Agricultural Institut by CV Mitra Tani Farm.

Plant height was measured at the age of 7 – 35 DAP once a week from the base of the stem to the highest part of the plant. Leaf area observation was carried out at 35 DAP (after harvest) and measured using the gravimetric method. Leaf color observation was done once at 35 DAP (at 7 AM just before the plants were harvested) using Munsell Plant Tissue Color Book. Observation of plant fresh weight was carried out by weighing plant samples from each treatment immediately after the plants were harvested, the whole parts of the plant including damaged parts were weighed using a digital scale.

The data obtained were analyzed using variance analysis (ANOVA). If there was a treatment effect, continue with the Duncan Multiple Range Test (DMRT) at the 5% level. The data processing used the DSAASTAT program).

PSB application was started by preparing isolates. PSB isolates were the collection of Biotechnology Laboratory UIN Bandung originating from soil post-excavation of Cimareme rocks, West Bandung district. The isolates were grown in shaken liquid Pikovskaya media for 3 x 24 hours using shaker at a speed of 150 rpm. Then, it was grown on solid Pikovskaya agar media in a petri dish for serial dilutions up to 10^9. Bacteria in petri dishes were incubated upside down for 3 x 24 hours at room temperature. All of these stages were carried out aseptically in Laminar Air Flow (LAF). The growing bacterial colonies were counted using the Total Plate Count (TPC) method with the Colony Counter tool. A total bacterial population of 30 x 10^6 CFU g^{-1} was obtained with varying bacterial sizes and clear zone (halo zone) sizes.

Bacteria that grew using the streak plate method with a total bacterial population of 30 x 10^6 CFU g^{-1} were taken entirely using ose needle and inoculated into Pikovskaya broth media in a glass bottle and shaken using a shaker for 3 x 24 hours. Next, the PSB isolate was diluted in mineral water according to the water content of the field capacity, which was 1 L, and applied according to the treatment, namely 5 mL, 10 mL, 15 mL, and 20 mL per polybag by pouring it evenly onto the ground in the polybag. The application of PSB was carried out 4 weeks after the application of drum fertilizer.

The post-excavated rock soil, which had been applied with organic material 3 times during three growing seasons, was sieved using a 0.5 cm soil sieve. Then, put it in a polybag measuring 30 cm x 30 cm, each polybag contains 4 kg of soil. Seeds were sown for 14 days in seedling trays or until the plants had three leaves. The seedling tray was filled with husk charcoal planting medium. Two seeds of bok choy were planted for each hole of the seedling tray. The manures used consisted of cow, goat, and laying hen manure, each of which was 15 t ha^{-1} (30 g polybag $^{-1}$). Organic manure fertilization was carried out 4 weeks before PSB application. Manure application was done by mixing the fertilizer with the soil in a polybag until evenly mixed.

Inorganic fertilizers used were Urea, SP-36, and KCl. Bok choy planting inorganic fertilization dosage refers to Haryanto (2007). SP-36 and KCl fertilizers were given 3 days before planting at half dose of 100 kg ha^{-1} (0.1 g $polybag^{-1}$) and 50 kg ha^{-1} (0.1 g $polybag^{-1}$) respectively, while urea was given 10 days after planting at a dose of 100 kg ha $^{-1}$ (0.2 g $polybag^{-1}$). The fertilizers were spread around the planting area.

Bok choy plant seedlings from the nursery were transferred to polybags measuring 30 cm x 30 cm with a planting hole depth of ± 5 cm, one seedling for each polybag. Planting distance between polybags was 20 x 20 cm. The planting was carried out at 06.00 AM to avoid wilting of the plants.

Plant maintenance included watering, re-planting, weeding, and pest and disease control. Watering was done at 07.00 AM and 05.00 PM, but the watering would be skipped for the rain. Replanting was carried out up to 7 DAP to replace bok choy plants that did not grow perfectly. Weeding was done manually by removing weeds that grew around the planting zone every 3 days from 7 – 35 DAP. Pest and disease control was carried out mechanically, namely by picking them up by hand and throwing them away from the research location.

The plants were harvested at the age of 35 DAP with the following harvest criteria: mature leaves with semi-round (oval) and wide, the leaf stalks were dark green, and the shape and size were relatively short. Harvesting was done by removing all parts of the plant from the ground.

Results and Discussion

Initial Soil Analysis. Based on the results of post-excavation soil analysis, the rock used had a sand fraction of 56%, 22% dust, and 22% clay (sandy clay texture class). This soil texture is relatively suitable for bok choy plants (Fatima et al., 2022). From the chemical properties of the soil, it was found that N was classified as very low (0.06%), organic C was very low (0.49%), available P was very high (73.0), and total P was classified as high (86.83 ppm) (Table 1).

Table 1. Soil analysis after rock excavation in Cimareme District.

Parameters	Value	Explanation
Sand, dust, clay (%)	56%, 22%, 22%	Sandy Clay Loam
pH	6.7	Neutral
C-organic (%)	0.49	Very low
N (%)	0.06	Very low
P-available (ppm)	73.0	Very high
P-total (mg 100g $^{-1}$)	86.83	Very high
K-available (ppm)	318.8	Very high
K-total (mg 100g $^{-1}$)	45.13	Very high
CEC	29.31	Tall

Description: Results of soil analysis at the laboratory of the Agricultural Instrument Standardization Agency (BSIP) for vegetable Crops, Lembang, West Bandung Regency

Table 2. Analysis of pH and nutrient contents of manures.

Parameters	Cow	Goat	Laying Hens	*Quality Standards
pH	8,11	6.3	8.8	4-9
C-organic (%)	40.52	43.21	19.51	Min.15
N (%)	1.33	1.03	2.24	Min.2
P_2O_5 (%)	0.45	0.54	4.27	Min.2
K_2O (%)	0.41	1.21	2.27	Min.2

Description: Analysis results based on manure content tests at the soils science and land resources laboratory, Bogor Agricultural Institute (IPB) (2022) and at the Agricultural Instrument Standardization Agency (BSIP) Laboratory for Vegetable Crops, Lembang, Bandung Regency (2023). *Quality standards based on RI Minister of Agriculture Decree No.261/KPTS/SR.310/M/4/2019.

Table 3. Effect of PSB and types of manure on plant height.

PSB	Average Plant Height (cm)				
	7 DAP	**14 DAP**	**21 DAP**	**28 DAP**	**35 DAP**
m_0 (0 mL polybag^{-1})	3.27 a	8.32 a	14.72 a	16.61 a	17.61 a
m_1 (5 mL polybag^{-1})	3.23 a	7.10 a	14.32 a	16.56 a	18.02 a
m_2 (10 mL polybag^{-1})	2.38 a	7.37 a	14.48 a	17.09 a	18.49 a
m_3 (15 mL polybag^{-1})	3.93 a	8.34 a	15.71 a	17.16 a	18.01 a
m_4 (20 mL polybag^{-1})	3.00 a	6.66 a	13.53 a	15.90 a	17.80 a
Manure (15 t ha^{-1}/ 30 g polybag^{-1})					
p_1 (cow)	2.77 a	7.43 a	13.64 a	16.47 a	17.68 a
p_2 (goat)	3.53 a	8.30 a	15.22 a	17.11 a	18.29 a
p_3 (laying hen)	3.18 a	6.95 a	14.79 a	16.41 a	17.99 a

Note: The average numbers followed by the same letter show no significant difference according to Duncan's advanced test at the 5% level.

Plant Height. The application of PSB and various types of manure did not show an independent or interaction effect on plant height at 7, 14, 21, 28, and 35 DAP (Table 3). PSB independently had no significant effect on plant height. This was related to the low bacterial population (30 x 10^6 CFU g^{-1}). This population does not yet meet the quality standards for biological fertilizer according to the Republic of Indonesia Minister of Agriculture Decree No.261/KPTS/SR.310/M/4/2019, namely 1 x 10^8 CFU g^{-1}. Apart from the low bacterial population at the time of application, the very high available P content in the initial soil (Table 1) can reduce the bacterial population. Suparnorampius et al. (2020) stated that P-available in the soil has the opposite effect on the population of PSB. The rhizosphere of mustard plants with P-available levels of 15.03 ppm produced a bacterial population of 9 x 10^4 CFU g^{-1}, while the carrot rhizosphere with an available P level of 12.11 ppm produced a higher bacterial population, namely 10 x 10 ^{4} CFU g^{-1}. Chapelle (2001) also explains that the growth of microorganisms is influenced by the availability of ready-to-use phosphorus compounds in their habitat. Too much available P can inhibit the activity of PSB itself which has an impact on reducing the bacterial population. Furthermore, bacterial populations are related to the amount of organic material that can be decomposed, as well as the mineralization of organic material to become inorganic so that it can be utilized by plants (Octaprama et al., 2020). Thus, a decrease in the PSB microbial population can have an impact on not maximizing the organic material from manure that can be used by plants to support plant height.

The variety of manure independently did not have a significant effect on plant height. This was because the nitrogen (N) content in manure generally cannot meet the needs of plants. The N requirement for bok choy plants according to Uchida (2000) is N of 2.39% (239 ppm). This amount is still above that provided by the manure used, namely cow manure only provided N of 1.33% (133 ppm), goat manure 1.03% (103 ppm), and chicken manure 2.24% (224 ppm). This means that the plants were still lack the nutrient N by 0.15 – 1.2%. According to Wijaya et al. (2022) leafy vegetable plants, especially annual plants such as bok choy, require large amounts of N to increase the production of amino acids in the plant body. N functions in the division of cells in meristem tissue which causes stem elongation resulting in an increase in plant height. Lack of N elements in this case causes the formation of vegetative organs to be hampered, especially stems which determine plant height because the photosynthate results obtained are not optimal (Pramitasari et al., 2016).

Apart from the nutrient N, plant height is also influenced by the element P. The element P is needed by plants for the formation of new cells in growing tissue, as well as strengthening stem reproduction (Rahmawati et al., 2019). In this study, the availability P is suspected to come from the work of microbes contained in manure as stated by Chen et al. (2022) that manure will increase soil microbes, then these microbes promote the transformation of moderately labile P (M-P) to labile P (L-P). This is in line with Brucker et al. (2020) that microbes released exopolysaccharides and siderophores that both work as chelator and manure provided as C source

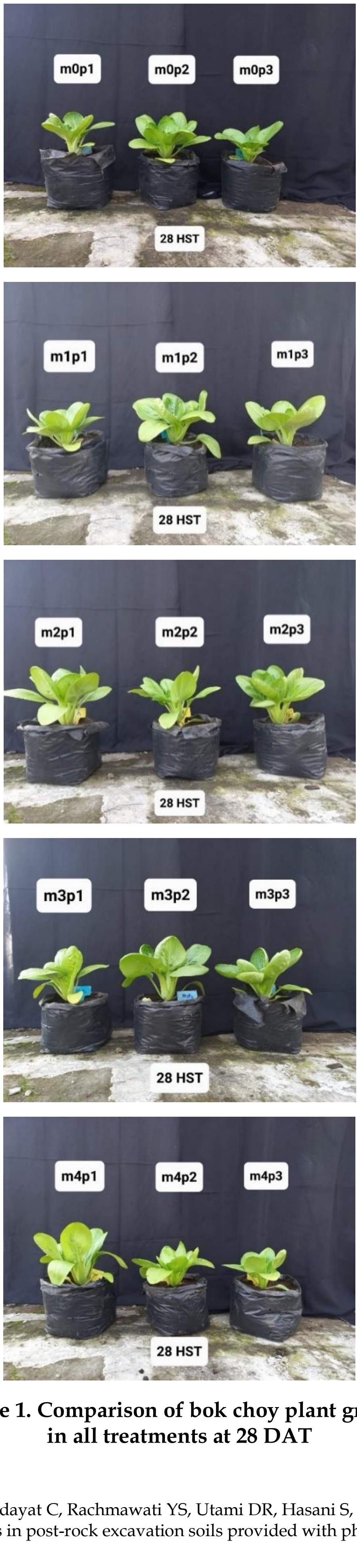

Figure 1. Comparison of bok choy plant growth in all treatments at 28 DAT

to make P solubilization rates increase. Excess P elements in plants can reduce plant height. This is in line with research (Firmansyah et al., 2015) that excess P causes the height growth of mangosteen plants to be hampered. The high level of P nutrients causes an antagonistic effect which causes a reduction in other nutrients, namely Zn and Fe (Sukmasari et al. , 2016). Zn plays a role in auxin synthesis with cell wall development and cell differentiation (Sivaiah, 2013). Meanwhile, Fe is responsible for activating enzymes and is a protein component that can stimulate plant height growth (Yolanda et al., 2020) . Therefore, the lack of these two elements due to high P is a supporting factor causing plants to grow stunted.

Leaf Area. Application of PSB and various types of manure fertilizer in interaction did not affect leaf area. Independently, the dose of PSB isolate also had no significant effect on leaf area, but the variety of manure had a significant effect on leaf area (Table 4). The application of various types of manure independently had a significant effect on increasing leaf area. Providing manure from laying hen produced the highest leaf area, namely 199.56 cm^2, this value was significantly higher to treatment with cow manure (134.34 cm^2) and goat manure (141.52 cm^2). The increase in leaf area is closely related to the availability of the nutrient N in plants as a form of plant vegetative organs. The N nutrient contained in laying hen manure, namely 2.24% (224 ppm), was not able to meet the N needs of plants, the same as the N element in cow manure 1.33% (133 ppm) and goats 1, 03% (103 ppm). Meanwhile, the N requirement for bok choy plants according to Uchida (2000) is 2.39% (239 ppm). However, if it is seen other factors, namely the C-organic value, laying hen manure had the lowest C-organic value, namely 19.51%. With this value divided by the N value (Table 1), a lower C/N ratio was obtained. This means that when applied, the laying hen manure had a better level of maturity than cow and goat manure, so the N nutrient contained in the laying hen manure was more readily absorbed by plant roots. According to Amnah & Friska, (2019) one of the components of plant tissue is carbon (C), during the composting process, carbon is used by bacteria as an energy source in constructing microbial cells by releasing CO_2 and other materials that evaporate. Therefore, there is a decrease in organic C levels in manure due to the breakdown that occurs during composting. The lower the C-organic content of manure indicates the better

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decomposition process carried out by microorganisms during the composting process. High C-organic in cow (40.52%) and goat (43.21%) manure implies that the composting process of organic material continues when the fertilizer is applied to the soil, and in these conditions, it is possible to reduce nutrient levels that plants need. The greater availability of organic compounds needed by plants from laying hen manure, especially N, causes the leaves to form wider than the other two treatments of cow and goat manure.

Table 4. Effect of PSB and Types of Manure on Leaf Area

PSB	Average (cm^2)
m_0 (0 mL $polybag^{-1}$)	147.27 a
m_1 (5 mL $polybag^{-1}$)	175.92 a
m_2 (10 mL $polybag^{-1}$)	147.04 a
m_3 (15 mL $polybag^{-1}$)	140.30 a
m_4 (20 mL $polybag^{-1}$)	181.85 a
Manure	
p_1 (cow 15 t ha^{-1})	134.34 a
p_2 (goat 15 t ha^{-1})	141.52 a
p_3 (laying hen 15 t ha^{-1})	199.56 b

Note: The average numbers followed by the same letter show no significant difference according to Duncan's advanced test at the 5% level.

The N element released by organic materials plays a role in compiling nucleic acids and proteins involved in the formation of chlorophyll (Widiyawati et al., 2014). If the plant's N needs can be met, more chlorophyll will be formed. According to Rachmadhani et al. (2018) if a large amount of chlorophyll is formed, more sunlight can be absorbed, the rate of plant photosynthesis increases, and ultimately the yield of photosynthesis also increases (Garfannsa et al., 2021) . In this research, the N content in various types of manure was still relatively low and could not meet the needs of plants. The phenomenon of no difference in leaf area between treatments due to N deficiency was also proven in the color parameters of the bok choy leaves produced. Bok choy leaves experienced pale leaves turning yellowish green which is an indication that the plant lacks the nutrient N.

Leaf Color. Determining leaf color refers to the standard colors contained in the Munsell Plant Tissue Color Book (MPTC). Color in this book consists of 3 variables, namely hue, value, and chroma. Hue is the dominant color of the spectrum according to wavelength. Value describes how light or dark a color is, while chroma describes how weak or strong a color is (Liana et al., 2023). The hue scale runs vertically from the lightest, with a value of 2.5 (at the top), to the darkest, with a value of 5 (at the bottom). The hue in all treatments in the study showed that the leaves were Green Yellow (GY) with the most intense level of yellowish green. The value scale is also read vertically, the further down it is, the brighter it is. The value for all treatments has a value of 7, which is the second highest value. This means that bok choy leaves are very bright. Meanwhile, the chroma scale runs horizontally, moving from weak (left) to strong (right), chroma in all treatments has values of 6 and 8, which means it is classified as strong (Table 5).

Table 5. Effect of PSB and Manure Variety on Leaf Color

PSB	Leaf Color
m_0 (0 mL $polybag^{-1}$)	5GY 7/6
m_1 (5 mL $polybag^{-1}$)	5GY 7/8
m_2 (10 mL $polybag^{-1}$)	5GY 7/6
m_3 (15 mL $polybag^{-1}$)	5GY 7/6
m_4 (20 mL $polybag^{-1}$)	5GY 7/6
Manure	
p_1 (cow 15 t ha^{-1})	5GY 7/6
p_2 (goat 15 t ha^{-1})	5GY 7/6
p_3 (laying hens 15 t ha^{-1})	5GY 7/6

Note: GY = Green Yellow ; 5GY = Hue ; 7/6 = Value/Chroma

The color of the bok choy leaves in all treatments at the end of the observation produced a relatively uniform color, namely yellowish green. The color of the leaves from this observation was lighter than the color stated in the description of the bok choy plant, where the color should be dark green. Pale leaf color is synonymous with a lack of nitrogen. This is in line with the results of the initial soil N analysis which was very low at 0.06% (6 ppm) and manure which was still below the needs of plants, where the N contained in cow manure was 1.33% (133 ppm), goat manure 1.03% (103 ppm), and laying hen manure 2.24% (224 ppm). On the other hand, according to Uchida (2000) bok choy requires a minimum N of 2.39% (239 ppm) resulting in a shortage of N elements of 0.15 – 1.2%. The nitrogen element forms chlorophyll which plays a role in giving leaves the green color (Setyanti et al., 2013). Therefore, N deficiency results in a color that is paler than the original color.

Apart from the lack of N, the color of the leaves turning yellowish green was thought to be caused by the high accumulation of K. The K nutrient available in the initial soil was 318.8 ppm, which is classified as very high. Next, the source of K nutrients in plants was also obtained from cow manure at 0.41% (41 ppm), goat manure at 1.21% (121 ppm), and laying hen manure at 2.27% (227 ppm) as well as fertilizer KCl dose was 100% (6 ppm), so that the K element available to plants was in excess by 0.7 – 2.2%. Element K is antagonistic to the element magnesium (Mg). Ding et al. (2006) stated that an antagonistic effect between K and Mg will appear if the K element is given in excess to plants, resulting in low Mg availability. It is important to know that not only nitrogen but Mg also plays an important role in the availability of chlorophyll in leaves.

According to Garfannsa et al. (2021) the green substance in leaves (chlorophyll) is also formed by the element Mg. Mg is the only metal ion contained in chlorophyll. Therefore, low Mg due to excess K can reduce chlorophyll content and also have an impact on the photosynthesis process of plants. This has been proven in research which shows that the application of 225 kg ha^{-1} KCl fertilizer produces a lower average chlorophyll content in sweet potato leaves, namely 920 µm fresh $weight^{-1}$ compared to the 78 kg ha^{-1} treatment which can produce higher chlorophyll, namely reaching 1060 µm fresh $weight^{-1}$. It can be concluded that chlorophyll is formed by the presence of the elements N and Mg. In this study, these two elements were insufficient in number.

Plant Fresh Weight. The application of PSB and various organic materials did not show any interaction or independent effects on plant fresh weight. Based on the research results, the fresh weight of bok choy plants obtained ranged from 75.0 – 91.4 g plant $^{-1}$ (Table 9). The fresh weight values were still below the plant description. The description states that the crop yield that can be obtained is 30 tons ha^{-1} or the equivalent of 120 g of $plants^{-1}$.

According to Mariay et al. (2023) the water content in plants will increase in line with the increase in nitrogen (N) content, thereby increasing the fresh weight of a plant. This happens because nitrogen plays a role in the formation of vegetative organs, namely leaves, stems, and roots. The higher and heavier the vegetative organs, the heavier the fresh weight of the plant. This is supported by Manuhuttu et al. (2018) who stated that fresh weight is the increase in the number of organs such as leaves, stems, and roots, which is influenced by the water content and nutrient content in plant tissue cells. In general, the N needs of bok choy plants were not met, so the formation of vegetative organs was hampered, the plants were stunted and the organs produced were small, as a result, the fresh weight of the plants was low.

Table 2. Effect of PSB and manure variety on plant fresh weight.

PSB	Average (g)
m_0 (0 mL $polybag^{-1}$)	91.4 a
m_1 (5 mL $polybag^{-1}$)	81.7 a
m_2 (10 mL $polybag^{-1}$)	84.7 a
m_3 (15 mL $polybag^{-1}$)	82.4 a
m_4 (20 mL $polybag^{-1}$)	75.9 a
Manure	
p_1 (cow 15 t ha^{-1})	75.0 a
p_2 (goat 15 t ha^{-1})	90.9 a
p_3 (laying hen 15 t ha^{-1})	83.8 a

Note: The average numbers followed by the same letter show no significant difference according to Duncan's advanced test at the 5% level.

The fresh weight of plants in relation to water content is also influenced by the availability of the element potassium (K). Potassium plays a role in regulating plant turgor pressure and osmosis. According to Wijaya et al. (2022) the element K can be absorbed by plants in the form of K^+ ions and plays a role in encouraging water absorption. Water absorption begins with the absorption of water by guard cells, which then causes an increase in cell turgor pressure which encourages the opening of the stomata. Open stomata provide a pathway for CO_2 and water to enter the plant so that the rate of photosynthesis increases. Furthermore, photosynthesis is used by plants for growth. According to Kurniawan et al. (2017) Sufficient K availability makes water use more efficient and maintains turgor, thereby facilitating metabolic processes. In this study, there was an excess of K. According to Uchida (2000), the K requirement for bok choy plants was 2.86% (286 ppm). The available K nutrient in the post-rock excavation soil was 3.188% (318.8 ppm), exceeding the plant K requirement of 0.328% or 38.2 ppm, and the excess nutrient value increases to 0.47% - 1.92% if K is accumulated with fertilizer cages and inorganic fertilizer. Besides that, the soil used has a pH of 6.7 (neutral). According to Silahooy et al., (2022) , K^+ ions are widely available in soil with a neutral pH. The very high availability of the K element can be antagonistic to the secondary macronutrient, namely Ca. As a result, the Ca^{2+} element will be retained and replaced by K^+ ions, resulting in low Ca availability. Ca plays a very important role at the root growth point. Ca deficiency causes stunted root formation and growth (Muchlis, 2017). Inhibition of root growth results in low plant water and nutrient absorption (Kurniawan et al., 2017), so that plant fresh weight is low or not significantly different between treatments.

Conclusion

Based on the research results, it can be concluded that:

1. There was no interaction between the dose of Phosphate Solubilizing Bacteria (PSB isolate and various types of manure (cow, goat, and laying hen) in increasing the growth and yield of bok choy (*Brassica rapa* L.) plants on post-rock excavation soil.
2. The dose of PSB isolates and various types of manure had not been able yet to increase the growth and yield of bok choy (*Brassica rapa* L.) plants on post-rock excavation soil mainly due to the soil natures.

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Yield comparison of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems

Abstract. Melon is one of the horticultural commodities with considerable economic potential. Cultivation through hydroponic systems can provide efficiency and increase production if complemented by superior varieties. This study aims to identify the best hydroponic system and melon variety combination with the highest yield. The research was conducted from November 2023 to April 2024 at the screen house in Pasir Kulon Village, Karanglewas District, Banyumas Regency, at 110 m above sea level. The study used a split-plot factorial design with a basic design of a Completely Randomized Block Design. The first factor was the hydroponic system, consisting of S1 = Dutch bucket and S2 = drip irrigation. The second factor was the variety, consisting of V1 = Golden Aroma, V2 = Rangipo, and V3 = Sweet Net. The results showed that the best yield was found in the Dutch bucket system and the Golden Aroma variety, with a fruit flesh thickness of 3.3 cm and a total soluble solids content of 17.18 °Brix.

Keywords: Dutch bucket system · Drip irrigation · Melon · Superior varieties

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Introduction

Horticulture is one of the new sources of agricultural growth expected to support national economic development. One of the horticultural products with potential is melon, or *Cucumis melo* L. Melon is an annual fruit plant that belongs to the gourd family or *Cucurbitaceae*. The fluctuating production of melon in Indonesia is one of the limiting factors; during the harvest season, the product is available in abundance, while outside the harvest season, it is scarce in the market, leading to price increases (Trisnawati *et al*., 2018). Sustainable production must be increased through hydroponics and superior varieties to meet the demand for melons.

The Dutch bucket system (DBS) is a hydroponic method that uses buckets connected to a drip irrigation system. According to Bhat *et al*. (2023), this system utilizes individual containers filled with inert growing mediums, ensuring stability and aeration. The drip irrigation delivers precise amounts of water and nutrient solutions directly to plant roots, re-circulating excess solutions to minimize waste.

The principle of drip irrigation is to provide water and nutrients through drops that drip periodically according to the plant's needs through a PE pipe with the help of a pressure pump (Sumarni *et al*., 2023). According to Wang *et al*., (2020), a drip irrigation system can save water use due to evapotranspiration reduction. Droplets on the system are directed right at the plant's root area so that the plant can directly absorb the water and nutrients provided. Drip irrigation systems can be divided into recirculating and nonrecirculating drip irrigation. Recirculating drip irrigation is a system that utilizes runoff nutrient solutions to be reused as nutrients for plants. Meanwhile, nonrecirculating drip irrigation is a system that does not reuse runoff solutions as plant nutrients.

To enhance production, selecting the right varieties is crucial. Variety plays an important role in producing yield because different varieties respond differently regarding genotypic characteristics, input requirements, growth processes, and the prevailing environment during the growing season (Rahman *et al*., 2010). The superior melon varieties Golden Aroma, Rangipo, and Sweet Net were selected for their distinct characteristics and benefits, including fruit appearance, weight, texture, and sweetness. This experiment tested two hydroponic systems, Dutch bucket and drip irrigation, on three melon varieties: Golden Aroma, Rangipo, and Sweet Net. The goals were to identify the best hydroponic system for melon growth and yield, determine the top-performing variety, and find the optimal combination of system and variety.

Material and Methods

The materials used were melon seeds (Golden Aroma, Rangipo, Sweet Net), rock wool, water, AB Mix, cocopeat, pesticide, and polybags. The equipment comprised the Dutch bucket and drip irrigation systems, net pots, seedling trays, TDS meter, measuring tape, refractometer, pruning shears, digital scale, string, sprayer, and thermohygrometer. The research occurred in a screen house in Pasir Kulon Village, Karanglewas District, Banyumas Regency, 110 meters above sea level, from November 2023 to April 2024. A split-plot factorial design with a Randomized Complete Block Design (RCBD) was used. The first factor was the hydroponic system (S1 = Dutch bucket, S2 = drip irrigation), and the second was the variety (V1 = Golden Aroma, V2 = Rangipo, V3 = Sweet Net). This design resulted in 6 treatment combinations, each replicated 4 times.

The Dutch bucket and drip irrigation systems had PVC pipes spaced 50 cm x 50 cm between each bucket/polybag. The Dutch bucket system used a nutrient solution, while the drip irrigation system employed cocopeat soaked in pond water for 2 weeks to reduce tannin levels. Melon seeds were germinated for 12 days before being transplanted into the hydroponic systems. Plant care involved tying plants to strings, pruning non-fruit-bearing lateral shoots and tips, pollination, fruit selection, sanitation, and pest and disease control. Fruits were harvested when physiologically ripe approximately 80 to 95 days after transplanting following seed producer recommendations.

Nutrient levels were monitored using a TDS meter, and the solution was adjusted based on plant needs and growth stage. Measured variables included plant height, number of leaves, leaf area, flowering age (male and female), fresh fruit weight, fruit diameter, fruit flesh thickness, total dissolved solids, root length, fresh root weight, and dry root weight. Data analysis was conducted using an Analysis of Variance (ANOVA) at a 5% significance level, with further analysis by Duncan's Multiple Range Test (DMRT) if significant variance was found.

Result and Discussion

General Condition. The screen house's daily temperature and humidity were recorded from 11:00 AM to 1:00 PM WIB, showing an average temperature of 33.5°C (range: 29 – 37.7°C) and humidity of 59.96% (range: 38 – 84%). These conditions are unsuitable for optimal melon production, which thrives at 20 – 30°C and humidity levels of 70 – 80% (Bambang *et al.*, 2021). Such unfavorable conditions can hinder growth and yield. The site, located approximately 110 meters above sea level, has a climatic context affecting these levels.

Plant Height. The hydroponic system showed no significant difference in plant height 8 weeks after planting. Average plant height growth is detailed in Table 3. Differences in plant height are primarily attributed to genetic variations among the three melon varieties, which affect their growth ability. According to Lenroot & Giedd (2011), these genetic differences lead to varied responses to environmental conditions, resulting in different growth outcomes.

Number of Leaves. Significant variations in the number of leaves at 8 weeks after planting were observed due to differences in hydroponic systems and varieties. The highest average number of leaves was recorded with the drip irrigation treatment (33.17 leaves) and the Rangipo variety (32.96 leaves) (Table 3). Genetic variations among varieties likely contributed to differences in leaf number. The drip irrigation system was most effective, likely because the nitrogen-rich cocopeat medium promoted vegetative growth. Drip irrigation enhances nutrient supply and plant needs in melon cultivation; according to Cabello et al. (2009), relationships between crop yield and water supply allow field quantification of water use efficiency in a given environment and can be assessed by developing local crop yield/water production functions.

Leaf Area per Leaf Blade. The hydroponic system treatments did not significantly affect leaf area, but there were significant differences among varieties at 8 WAP. The Sweet Net variety had the largest leaf area (274 cm^2), while the Rangipo variety had the smallest (209.43 cm^2) (Table 3). The lack of significant difference between hydroponic systems is likely due to genetic factors and environmental conditions, such as sunlight. According to Rezai *et al*. (2018), shade plants develop larger and thinner leaves to increase light harvest.

Flowering Age. The hydroponic system did not significantly affect flowering age, but significant differences were observed among varieties. The Rangipo variety had the earliest male (14.75 days after planting) and female (21.31 days after planting) flowering, followed by Golden Aroma, and the Sweet Net variety flowering the last (Table 4). Varietal differences lead to differences in flowering time. The duration of flowering time and genome size are positively correlated (Bilinski et al., 2018). Flowering speed is influenced by variety and genetic factors. Attribute differences in flowering age to genetic influences and environmental conditions. According to Takeno (2016), many types of stress factors have been reported to induce flowering. These include high or low light intensity, UV light, high or low temperature, poor nutrition, nitrogen deficiency, drought, low oxygen, crowding, root removal, and mechanical stimulation. The average temperature during the experiment was 33.5°C, higher than the optimal 20 – 30°C, which likely accelerated flowering. Higher temperatures speed up flowering, while unsuitable temperatures can reduce enzyme activity involved in flowering; the rate of every biochemical reaction depends on it; ambient temperature also accelerates or delays flowering time (Takeno, 2016).

Table 1. Comparison of quantitative phenotypic characteristics among three melon varieties.

Parameter Description of Varieties	Measurement Results		
	Golden Aroma	Rangipo	Sweet Net
Leaf length (cm)	7.7 – 17.5	8.7 – 15.6	12.1 – 14.7
Leaf width (cm)	10.2 – 22.5	12.2 – 22	16.6 – 20.5
Male flowering age (days after planting)	14 – 18	12 – 16	15 – 20
Female flowering age (days after planting)	18 – 25	16 – 24	20 – 26
Weight per fruit (g)	913.3 – 1963	683.3 – 991.3	812.3 – 973.7
Horizontal fruit diameter (cm)	12.63 – 15.57	11.25 – 13.42	10.96 – 12.45
Vertical fruit circumference (cm)	37 – 53	32 – 44.7	36 – 45.5
Horizontal fruit circumference (cm)	42.4 – 50.3	31.5 – 42.5	33.5 – 39
Fruit rind thickness (cm)	0.2 – 0.4	0.2 – 0.3	0.1 – 0.2
Fruit flesh thickness (cm)	3 – 3.3	3.2 – 3.23	2.8 – 3
Total soluble solids (°Brix)	15 – 17	14– 15	14 – 16

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Table 2. Comparison of Qualitative Phenotypic Characteristics among Three Melon Varieties

Parameter Description of Varieties		Golden Aroma	Rangipo	Sweet Net
Stem	Shape	Cylinder	Cylinder	Cylinder
	Color	Green (RHS N144C)	Green (RHS 145A)	Green (RHS 145B)
Leaf				
	Shape	Triangular shape (triangularis)	Triangular shape (triangularis)	Triangular shape (triangularis)
	Color	Dark green (RHS 138B)	Dark green (RHS 137A)	Dark green (RHS 138A)
Flower				
	Shape	Rotate	Rotate	Rotate
	Flower petal color	Yellow (RHS 12A)	Yellow (RHS 12B)	Yellow (RHS 12B)
Fruit				
				
	Shape	Round/Globular	Round/Globular	Elongated/Oval
	Fruit skin color	Dark green (RHS 137A)	Light green (RHS 192D)	Yellow (20D)
	Surface texture	Dense netting present	Dense netting present	Sporadic netting
	Fruit flesh color	Orange (RHS 28D)	Orange (RHS 29C)	Orange (RHS 29B)
	Fruit flesh crispness	Crisp	Crisp	Crisp
Seed	Shape	Ellipse	Ellipse	Ellipse
	Color	Yellow (RHS 18C)	Yellow (RHS 18C)	Yellow (RHS 18C)

Note: Classification based on Daryono & Nofriarno (2018).

Table 3. Average plant height, number of leaves, and leaf area of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Growth Variables at 8 Weeks After Planting (WAP)		
	Plant height (cm)	Number of leaves	Leaf area (cm^2)
Hydroponic system			
S1 (*Dutch bucket*)	246.92	29.69 b	236.10
S2 (Drip irrigation)	253.16	33.17 a	256.77
Varieties			
V1 (Golden Aroma)	249.86	32.08 b	255.76 b
V2 (Rangipo)	246.03	32.96 a	210.22 c
V3 (Sweet Net)	254.23	29.25 c	273.33 a
C.V. (%)	3.68	6.62	15.79

Note: Numbers followed by different letters in the same column indicate significant differences at the 5% DMRT.

Table 4. Average flowering age of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Flowering Age (Days After Planting - DAP)	
	Male	Female
Hydroponic system		
S1 (*Dutch bucket*)	16,58	21,96
S2 (Drip irrigation)	16	22,29
Varieties		
V1 (Golden Aroma)	16.42 b	21.88 ab
V2 (Rangipo)	14.75 a	21.31 a
V3 (Sweet Net)	17.71 c	23.19 b
C.V. (%)	8.40	4.23

Note: Numbers followed by different letters in the same column indicate significant differences at the 5% DMRT.

Fresh Fruit Weight. The hydroponic system treatments did not significantly affect fresh fruit weight, but significant differences were observed among varieties. The Golden Aroma variety had the highest average fresh fruit weight at 1570.96 g, followed by Sweet Net and Rangipo (Table 6). Genetic factors influence variations in fresh fruit weight. Fresh fruit weight is also affected by plant organs, such as leaves. Leaf area influences photosynthesis and plant growth by determining light interception through the leaf surface, morphology, and orientation. This is particularly important in noncontinuous canopies (e.g., young plants), where the incident light is only partially intercepted, and photo-morphogenetic responses have a relevant impact on plant growth and productivity (Paradiso & Proietti, 2022). The fresh fruit weight also rises when plant height and leaf area increase. This is evident in the Rangipo variety, which has the smallest leaf area and lower fresh fruit weight. This is due to less effective sunlight absorption, reducing photosynthesis and photo-assimilate production.

The yield potential of each variety influences the difference in fruit weight across each variety. The Golden Aroma variety has the highest average weight of 1570.96 g, while the seed producer claims that the yield potential of the Golden Aroma variety can reach up to 2500 g. The Rangipo and Sweet Net varieties have average weights of 883.58 g and 898.75 g, respectively, with the seed producer claiming that the yield potential of the Rangipo variety can reach up to 2000 g. The yield potential of Golden Aroma, as claimed by the producer, is higher than that of Rangipo and Sweet Net.

The two hydroponic systems did not show significant differences in fresh fruit weight, likely because both systems provided similar and adequate nutrient supplies. Plants with adequate nutrients will grow well. Phosphorus (P) in hydroponic nutrients plays a role in plant growth. According to Shirko *et al.* (2018), phosphorus deficiency is not very common in hydroponic systems compared to N and K deficiencies; however, it can greatly reduce yield and plant growth. Syah & Yulia (2021) stated that optimal phosphorus uptake increases and multiplies ATP in plants, which is needed for cell division and enhances photosynthetic output, thus improving fresh fruit weight.

Fruit Diameter. The hydroponic system treatments did not show significant differences in melon fruit diameter, while different varieties showed significant differences. Table 5 shows that the Golden Aroma variety had the highest average diameter at 14.15 cm, followed by the Rangipo and Sweet Net varieties. Fruit diameter is closely related to fresh fruit weight; as weight increases, diameter also increases. Some important traits (fruit weight, diameter, and length) indicated that they were quantitative traits controlled by multiple nuclear genes (Lian *et al.*, 2021).

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Table 5. Average fresh fruit weight and fruit diameter of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Observation Variables	
	Fresh Fruit Weight (g)	Fruit Diameter (cm)
Hydroponic system		
S1 (*Dutch bucket*)	1125.05	12.69
S2 (Drip irrigation)	1110.47	12.51
Varieties		
V1 (Golden Aroma)	1570.96 a	14.15 a
V2 (Rangipo)	883.58 c	12.12 b
V3 (Sweet Net)	898.75 b	11.5 c
C.V. (%)	18.12	7.24

Note: Numbers followed by different letters in the same column indicate significant differences at the 5% DMRT.

Several studies about the effect of pruning on carbohydrate content in peaches and cherries have been carried out and report similar findings (Clair-Maczulajtys *et al.*, 1994; Ikinci, 2014). The study pointed out that this practice reduced shoot length, stimulated shoot diameter enlargement, decreased fruit yield, and increased fruit weight; in addition, if applied, this technique increased significantly fruit soluble solids content (SSC). The increase in fruit size and quality has been attributed to higher photosynthate availability in the fruit of pruned trees due both to the increment in photosynthetic photon flux density (PPFD) and to the elimination of competing sinks, i.e., watersprouts. The improved light exposure, in turn, may increase fruit sink activity, thus positively affecting fruit size (Falchi *et al.*, 2020).

Fruit Flesh Thickness. The analysis of variance results shows that both hydroponic system treatments and different varieties resulted in significant differences in fruit flesh thickness, as presented in Table 6. The table shows that the best fruit flesh thickness, 3.3 cm, was achieved with the Dutch bucket system and Golden Aroma variety. This combination does not significantly differ from the Dutch bucket system with Rangipo or the drip irrigation system with Rangipo, but it does differ significantly from other treatments. The lowest flesh thickness, 2.83 cm, was found with the Dutch bucket system and Sweet Net variety. Fruit diameter and weight are closely related to flesh thickness, with larger fruit size corresponding to thicker flesh as assimilates are stored as food reserves in the fruit.

The formation of melon flesh is influenced by nutrients absorbed by the plants and transported by roots to all the plant organs. Some nutrients will also be transferred to developing fruit so that it will supply nutrients to produce fruit with optimal quality and quantity. The optimal supply of nutrients that are balanced from all nutrients guarantees the quantity and quality of the fruit to be harvested. The flesh thickness can also be influenced by sugar content, aroma, taste, fruit weight, and fruit volume (Sugiartini *et al.*, 20022).

Total Soluble Solids. The analysis of variance indicates significant effects of both hydroponic system treatments and variety differences on the total soluble solids in melon fruit, as presented in Table 7. The Dutch bucket system and Golden Aroma variety produced the highest total soluble solids at 17.18 °Brix. This was followed by the Dutch bucket system with the Sweet Net variety and the drip irrigation system with Golden Aroma, which were not significantly different. The lowest total soluble solids, 14.83 °Brix, were observed with the Dutch bucket system and Rangipo variety. The combination of the Dutch bucket system and Golden Aroma variety is optimal for total soluble solids, likely due to the variety's superior genetic traits and the consistent nutrient supply from the system, enhancing leaf area and photosynthesis and thus increasing sugar accumulation in the fruit. The differences in observational variables between melon varieties are more directed to the genetic factors of the varieties tested against a wide genetic diversity so that they have different variations, including fruit diameter, wet weight, and fruit sweetness level (Aragao *et al.*, 2013)

According to Falchi *et al*. (2020), thinning induces a transient accumulation of soluble sugars in the leaves, leading to reduced photosynthesis and stomatal closure without significantly impacting the final fruit size. The results indicate the influence of crop load on fruit-water relationships, possibly improved by frequent irrigation in un-thinned plants, allowing the fruit to reach its maximum potential (Andreade *et al.*, 2019).

Table 6. Interaction effect on fruit flesh thickness of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Fruit flesh thickness (cm)		
	V1 (Golden Aroma)	V2 (Rangipo)	V3 (Sweet Net)
S1 (*Dutch bucket*)	3.3 x	3.2 x	2.83 y
	a	a	b
S2 (Drip irrigation)	3 y	3.23 x	3 y
	b	a	a

Note: Numbers followed by the same letters in rows (x, y, z) and columns (a, b) indicate no significant differences at the 5% DMRT.

Table 7. Interaction effect on total soluble solids of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Total soluble solids (°Brix)		
	V1 (Golden Aroma)	V2 (Rangipo)	V3 (Sweet Net)
S1 (*Dutch bucket*)	17.18 x	14.83 z	16.73 y
	a	b	a
S2 (Drip irrigation)	16.13 x	15.08 z	15.4 y
	b	a	b

Note: Numbers followed by the same letters in rows (x, y, z) and columns (a, b) indicate no significant differences at the 5% DMRT.

Table 8. Average root length, fresh root weight, and dry root weight of three melon (*Cucumis melo* L.) varieties cultivated with dutch bucket and drip irrigation systems.

Treatments	Observation variables		
	Root lenght (cm)	Fresh root weight (g)	Dry root weight (g)
Hydroponic system			
S1 (*Dutch bucket*)	26.71 b	131.43 a	11.14 a
S2 (Drip irrigation)	39.88 a	28.98 b	3.36 b
Varieties			
V1 (Golden Aroma)	31.44	61.95 c	7.12
V2 (Rangipo)	34.94	65 b	6.7
V3 (Sweet Net)	33.5	113.65 a	7.95
C.V. (%)	12.26	41.02	67.27

Note: Numbers followed by different letters in the same column indicate significant differences at the 5% DMRT.

Root Length. The hydroponic system treatments showed significant differences in root length, whereas the different varieties did not show significant differences. Table 8 illustrates significant differences in root length between the two systems due to the different growing media used. The drip irrigation system produced the longest roots to the Dutch bucket system. The growing media is closely related to its support for root growth. The Dutch bucket system, with the medium filled with water to the height of the bucket, can influence the resulting root length. Pratiwi (2021), states that the height of the container can affect root growth; taller containers allow roots to grow longer in search of the nutrients and water required by the plants the plants require. The drip irrigation system with cocopeat media likely provides better oxygen circulation in the root area due to its air-filled pores, resulting in better root development and unimpeded plant growth. According to Phogat *et al*. (2015), a constant supply of O_2 is essential, and its concentration should be at least 10% for the plants to grow normally. A lack of O2 is more injurious to plants than an excess of CO2 within reasonable limits (<20%). An excess of O_2 is also undesirable because it oxidizes the organic matter rapidly and dries the soil quickly.

Cocopeat media contains many additional nutrients besides regular nutrient applications. These nutrients are crucial for plant growth and development, including macro and micronutrients such as potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium, and other minerals that support root elongation. Nurifah & Fajarfika (2020) state that cocopeat contains micro-nutrients

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like copper (Cu), which plays a role in electron transport during photosynthesis and root formation, and zinc (Zn), which supports root growth and leaf expansion.

Fresh Root Weight. The hydroponic system treatments and varieties resulted in significant differences in fresh root weight. Table 8 shows that the Dutch bucket system produced higher fresh root weights than the drip irrigation system. This is likely due to the abundant nutrient supply in the Dutch bucket system, which provides ample nutrients to the roots, promoting rapid development of lateral roots and thus increasing fresh root weight. The Sweet Net variety showed significantly higher fresh root weight than other varieties. Manuhuttu *et al*. (2014), explain that root system development is primarily controlled by the plant's genetic traits and the growing media conditions.

Dry Root Weight. The hydroponic system treatments showed significant differences in dry root weight, while the different varieties did not show significant differences. Table 8 indicates that the Dutch bucket system resulted in the highest dry root weight, significantly different from the drip irrigation system. This is suspected because dry root weight is proportional to fresh root weight. Manuhuttu *et al*. (2014), state that plants with higher fresh root weights also have higher dry root weights and root volumes, indicating that these plants are well-supplied with photosynthates. However, each variety has a different root water uptake rate that is genetically influenced. The BmBm gene controls the rate of water uptake from the soil and the incoming radiation so that the transpiration rate can be controlled (Aqil & Bunyamin, 2013).

Conclusion

The Dutch bucket system yielded the best results for total soluble solids, fresh root weight, and dry root weight. The drip irrigation system excelled in leaf number and root length. The Golden Aroma variety achieved the highest fruit weight and total soluble solids. The Rangipo variety showed superior growth in leaf number, flowering age, and fruit flesh thickness, while the Sweet Net variety excelled in leaf area and fresh root weight. The Dutch bucket system with the Golden Aroma variety was the best combination for fruit flesh thickness (3.3 cm) and total soluble solids (17.18 °Brix).

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Fitriatin BN · Ghifari RFH · Sofyan ET · Widiantini F · Fakhrurroja H · Simarmata T

The Role of nutrient solutions on phosphate-solubilizing bacteria population, phosphorus availability, phosphorus uptake, growth and yield of red chili (*Capsicum annuum* L.)

Abstract. Red chili consumption in Indonesia has increased every year. However, with large chili production to meet large consumption, land conversion for various purposes has reduced the harvested area. The efforts to increase the harvested area of chili using Inceptisols soil by providing nutrient solutions to overcome the infertility of the soil using its nutrients. This experiment aims to determine the effect of nutrient solution application on population of phosphate-solubilizing bacteria, phosphorus availability, phosphorus uptake, growth and yield of Red Chili (*Capsicum annuum* L.) in Inceptisols. The experiment was conducted from August 2023 to February 2024 at Ciparanje Experimental Field, Faculty of Agriculture, Padjadjaran University, and the analysis process was conducted at the Laboratory of Soil Biology, and Soil Chemistry and Plant Nutrition, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran., using a factorial randomized block design with two factors, nutrient solutions concentrates (1200, 1600, 2000 ppm) and nutrient solutions doses (200, 400, 600 mL), resulting in nine treatments and three replications. The results showed that the treatment of nutrient solution concentration and dose increased the number of fruits per plant, fruits weight per plant, and yield of chili with grade A. Treatment with 2000 ppm concentrate + 600 mL dose gave the best results on the number of fruits per plant (44.7 fruits), fruit weight per plant (725g), and grade A chili yield (73 fruits).

Keywords: Inceptisols · Nutrients · Phosphate · Red chilli

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Fitriatin BN, Ghifari RFH, Sofyan ET, Widiantini F, Fakhrurroja H, Simarmata T. 2024. The Role of nutrient solutions on phosphate-solubilizing bacteria population, phosphorus availability, phosphorus uptake, growth and yield of red chili (*Capsicum annuum* L.). Jurnal Kultivasi, 23(2): 354-363.

Introduction

Red chili plants are very important in cultivation because of the high demand for their products. Quoted from data from the Central Statistics Agency (2024), there was an increase in red chili production in 2022 to 2023. In 2022, the amount of large red chili production in Indonesia was 1,475,821 tons, which increased by 5.33% or 78,667 tons to 1,554,498 tons in 2023. The increase in the amount of large red chili production in Indonesia was accompanied by a decrease in the harvest area for this commodity.

The large number of land conversions, opening of industrial areas, and residential areas are factors causing the decrease in the area of agricultural harvest land in Indonesia (Ivanka et al., 2024). Stringer et al. (2020) stated that creating potential land by utilizing existing land with various methods such as urban farming can be a solution to overcome the decrease in the area of harvest land.

The land with a fairly large area in Indonesia is the Inceptisols soil order, with an area of around 40% of the total land area in the country (Muslim et al., 2020). In tropical countries, Inceptisols plains that experience drought will experience a decrease in organic matter levels, so that their fertility is relatively low (Suwardi, 2019). One effort to overcome this problem can be done by applying organic fertilizers along with inorganic fertilizers to improve soil fertility. One example of inorganic fertilizer is a ready-to-absorb nutrient solution.

Phosphorus is a nutrient that helps in biochemical and physiological reactions of plants (Khan et al., 2023). Vasconcelos et al. (2022) stated that phosphorus plays an important role in plant respiration, photosynthesis, plant metabolism, where phosphorus as an essential nutrient certainly helps to encourage plant growth. Malhotra, et al. (2018) stated that phosphorus for plants functions to help assimilation, accelerate seed ripening and flowering, and stimulate root growth. With the important role of phosphorus in plant growth and development, the lack of phosphate availability that can be absorbed by plants is a problem because only 0.01% of the total P solubility can be absorbed by plants (Bhat et al., 2024).

One way to increase the levels of dissolved phosphorus that can be absorbed by plants is by using Phosphate Solubilizing Bacteria (PSB). Phosphate solubilizing bacteria can make previously insoluble phosphate become dissolved and can be absorbed by plants. Fitriatin, et al. (2022) explained that phosphate solubilizing bacteria make the use of organic phosphorus fertilizer more efficient, which helps plants by increasing the concentration of plant phosphorus and overcoming the lack of phosphorus availability. Based on the description above, the purpose of this research to study nutrient solutions with the right concentration and dosage to increase the population of phosphate solubilizing bacteria, phosphorus availability, phosphorus absorption, and the productivity and yield of red chilies in soil media in polybags.

Materials and Methods

The materials were used red chili seeds of the F1 pillar variety, nutrient solution contain of macro and micronutrients, Inceptisols soil (pH organic of 6.05 (slightly acidic); organic carbon of 1.5% (low); total nitrogen of 0.23% (medium); potential phosphorus of 640.2 mg 100 g^{-1} (very high); phosphorus availability of 1.035 ppm (very low); cation exchange capacity of 23.78 cmol kg^{-1} (medium); and a population of phosphate-solubilizing bacteria 36 x 10^7 CFU mL^{-1} (medium), manure and biocar-based bioameliorant enriched with *Trichoderma* sp. The design used in this experiment is a Factorial Randomized Block Design. There were two factors in this design, with the first factor being the treatment of nutrient solution concentration (1200, 1600 and 2000 ppm) and the second factor being the dose of nutrient solution (200, 400 and 600 mL). There were nine treatment combinations, where each treatment was repeated in three replications. The nutrient solution is given every 3 days with a dose of nutrient solution according to the treatment.

The observations conducted in this study include primary and supporting observations. The primary observation variables in this research are as follows:

1. The population of phosphate-solubilizing bacteria in the soil was assessed using the total plate count (TPC) method with Pikovskaya medium. Observations were made during the destruction of plants at the end of the vegetative phase or at eight weeks after planting (WAP).
2. Phosphorus availability was measured using the Olsen method, with observations also

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conducted during plant destruction at eight WAP.
3. Phosphorus uptake was determined using the wet ash method, with measurements taken at the same time as plant destruction.
4. The dry weight of the plants was measured using the oven-drying method, also during plant destruction at eight WAP.
5. The growth of chili plants, including the number of leaves, plant height (cm), and stem diameter (mm), was observed every two weeks during the vegetative phase, from two to four WAP.
6. The yield of chili plants over eight harvests, including the number of fruits per plant and total fruit weight (g), was measured using scales and rulers at harvest time.
7. The quality of the chili yields (Grade A, B, C) was classified based on Indonesian National Standards and was assessed during harvest.

The data from the study were statistically analyzed using a Factorial Randomized Block Design (FRBD), employing the SPSS application to conduct a normality test to determine the normal distribution of the data. If the results of the normality test indicate a normal distribution, the analysis will proceed with analysis of variance (ANOVA) at a significance level of 5% to assess the effects of the treatments (whether they are significant or not). If the ANOVA results show a significant effect, it will be followed by Duncan's Multiple Range Test (DMRT) at a significance level of 5%.

Results and Discussion

Phosphorus Availability and Phosphate-solubilizing Bacteria Population. Available phosphorus is soil P that can be dissolved in water and citric acid. The form of P in the soil can be distinguished based on its solubility and availability in the soil, water-soluble P is the form of P that can be absorbed by plants.

Based on the results of the analysis of variance at a significance level of 5%, there was no interaction between the concentration and dosage treatments of nutrient solutions on phosphorus availability. This is suspected to be due to the binding of phosphorus with Al and Fe in the soil, as stated by Rahmayuni, et al. (2023), who noted that the Inceptisols contain clay (which includes Al and Fe) ranging from 21.7% to 36.6%, which can bind phosphorus. Additionally, the population of phosphate-solubilizing bacteria is thought to be affected by the application of nutrient solutions, which may lead to an abundance of nutrients, thereby causing phosphate-solubilizing bacteria to lack energy sources for proliferation (Pan and Cai, 2023). The independent effect of concentration in the nutrient solution treatment significantly influenced the population of phosphate-solubilizing bacteria.

Table 1. The effect of nutrient solutions on phosphorus availability and the population of phosphate-solubilizing bacteria.

Treatments	Phosphate Availability (ppm)	PSB population (x 10^7 CFU g^{-1})
Nutrient Solution Concentrations		
1200 ppm	24.10	29.72 a
1600 ppm	28.16	50.06 b
2000 ppm	31.64	60.72 b
Nutrient Solution Doses		
200 mL	27.89	38.22
400 mL	26.49	55.89
600 mL	29.52	46.39

Note: Numbers marked with the same letter indicate no significant difference based on the Duncan's Multiple Range Test at a significance level of 5%.

Based on Table 1, the treatment of nutrient solution concentration increases the phosphorus availability in the soil, although it does not have a significant effect. The nutrient solution concentration at each level is directly proportional to the increase in phosphorus availability in the soil, rising from 1200 ppm to 1.600 ppm (16.7%) and further increasing at 2000 ppm (12.3%). The dosage of the nutrient solution also non-significantly increased available P in the soil from 200 mL to 400 mL (5%), but decreased at 600 mL (11.4%). In the initial soil analysis, available P in the Inceptisols of Jatinangor was only 1.04 ppm P. The independent effects of nutrient solution concentration and dosage increased available P in the soil, but did not show significant differences. This is suspected to be due to the Inceptisols' inability to retain phosphorus effectively, leading to leaching of the nutrient, consistent with Ginting, et al. (2024), who stated that Inceptisols have low nutrient retention capacity.

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The treatment of nutrient solution concentration significantly increased the population of phosphate-solubilizing bacteria (PSB) in the soil, particularly at a concentration of 2000 ppm. However, this increase was not statistically different from the treatment at 1600 ppm. In the initial soil analysis, the population of PSB in Jatinangor Inceptisols was recorded at 36 x 10^7 CFU mL^{-1}. The nutrient solution concentration raised the PSB population from 1200 ppm to 1600 ppm (68.4%), and further increased at 2000 ppm (21.3%). The rise in PSB population is believed to be due to the presence of micronutrients and Fe-EDTA in the nutrient solution, which act as cofactors in the formation of phosphatase enzymes by PSB. This is consistent with the statement by Vigani and Murgia (2018) that iron can serve as a cofactor in enzymatic reactions involving PSB, thus contributing to the increase in PSB populations.

The application of nutrient solution doses did not significantly increase the population of phosphate-solubilizing bacteria (PSB) from a dose of 200 mL to 400 mL by 46.3%, but it decreased by 17% at a dose of 600 mL. This decline is suspected to be due to the high nutrient solution dose providing excessive nutrients, which may lead to insufficient energy sources for the PSB to proliferate. The growth of PSB can accelerate when they receive the necessary materials for their development. The addition of enriched biochar containing a mixture of dolomite and guano can enhance the population of PSB by increasing organic carbon content (Lai et al, 2022).

Dry Weight of Plant and Phosphorus Uptake. Based on the analysis of variance at a significance level of 5%, it was shown that there was no interaction between the concentration and dose treatments of the nutrient solution on the dry weight of the plants and phosphorus uptake. This is suspected to be because the nutrient supply from the nutrient solution was less than what was needed to release potential phosphorus (Johan et al., 2021), resulting in suboptimal plant growth during the vegetative phase. The independent effects of the concentration and dose treatments of the nutrient solution significantly influenced both the dry weight of the plants and phosphorus uptake.

Based on the data in Table 2, the treatment of nutrient solution concentration significantly increased the dry weight of the plants at a concentration of 1200 ppm, but there was no significant difference compared to the concentration of 1600 ppm. The dry weight of the plants decreased by 1.1 g (4.08%) from 1200 ppm to 1600 ppm, and decreased by 10.5 g (40%) from 1600 ppm to 2000 ppm. The application of nutrient solution doses increased the dry weight non-significantly by 2.2 g from a dose of 400 mL to 600 mL (9.91%). The significant decrease in dry weight at higher concentrations of the nutrient solution and the non-significant differences in dry weight due to the doses are suspected to be because the plants were unable to absorb excessive nutrients. Therefore, nutrient application during the vegetative phase tends to be more beneficial at lower concentrations of the nutrient solution. Plant growth, especially during the vegetative phase, is greatly influenced by the availability of nutrients and water, but it is also affected by the plant's ability to absorb them (Francis et al., 2023). Fertilization with nutrient solutions containing nitrogen can increase the number of leaves and leaf area in plants, which ultimately can enhance the dry weight of the plants.

Table 2. The effect of nutrient solutions on dry weight of plant and phosphorus uptake on eight weeks after plant.

Treatments	Dry Weight (g)	P Uptake (mg $Plant^{-1}$)	P Content (%)
Nutrient Solution Concentrations			
1200 ppm	27.22 b	80.81 ab	0.35
1600 ppm	26.11 b	103.41 b	0.43
2000 ppm	15.56 a	53.59 a	0.30
Nutrient Solution Doses			
200 mL	22.22	86.40	0.41
400 mL	22.22	68.27	0.32
600 mL	24.44	83.14	0.36

Note: Numbers marked with the same letter indicate no significant difference based on the Duncan's Multiple Range Test at a significance level of 5%.

The treatment of nutrient solution concentration significantly increased phosphorus uptake at a concentration of 1600 ppm, but there was no significant difference compared to the concentration of 1200 ppm. Phosphorus uptake increased by 27% from 1200 ppm to 1600 ppm and then decreased by 48% from 1600 ppm to 2000 ppm. This decline is suspected to be due to the plants' ability to absorb nutrients in small to moderate amounts, limited by their capacity for nutrient uptake (Alaoui et al., 2022). The application of

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nutrient solution doses decreased phosphorus uptake by 18.2 g from a dose of 200 mL to 400 mL but increased by 14.9 g at a dose of 600 mL. The phosphorus content obtained from the analysis did not show significant differences due to either the concentration or the doses of the nutrient solution.

The lack of significant effect of nutrient solution doses on phosphorus uptake and phosphorus content in plants is suspected to be due to a substantial amount of phosphorus being bound, which prevents optimal phosphorus uptake by the plants. The release of phosphorus by phosphate-solubilizing bacteria (PSB) greatly influences the availability of phosphorus in the soil, as well as its absorption by plants. The amount of phosphorus absorbed by plants is directly proportional to the dry weight of the plants, with phosphorus playing a crucial role in enhancing root growth, allowing plants to absorb more nutrients. This improved nutrient uptake contributes to better plant growth and increased biomass. Takahashi and Katoh, (2022) state that the contact between plant roots and phosphorus, along with the distribution of plant roots, significantly affects phosphorus uptake and the dry weight of the plants.

Chili Plant Growth. Based on the analysis of variance at a significance level of 5%, there was no interaction between the concentration and dose treatments of the nutrient solution on the number of leaves in chili plants. This is suspected by the vegetative phase, the plants were unable to absorb nitrogen effectively to significantly increase the leaf count. The independent effect of the dose treatment of the nutrient solution had a significant impact on the number of leaves at two WAP (weeks after planting).

Table 3. The effect of nutrient solutions on number of leaves.

Treatments	Number of Leaves		
	2 WAP	4 WAP	6 WAP
Nutrient Solution Concentrations			
1200 ppm	9,83	13.17	60.50
1600 ppm	9.50	15.00	75.44
2000 ppm	10.39	13.78	66.39
Nutrient Solution Doses			
200 mL	9.50 a	14.83	75.22
400 mL	9.67 ab	12.83	63.11
600 mL	10.56 b	14.27	64.00

Note: Numbers marked with the same letter indicate no significant difference based on the Duncan's Multiple Range Test at a significance level of 5%.

In Table 3, the best treatment concentration of the nutrient solution for increasing the number of leaves non-significantly was found at a concentration of 1200 ppm at two WAP, while the best treatment was at a concentration of 1.600 ppm at four and six WAP. This is suspected to be because, during the vegetative phase, the plants did not have the ability to absorb nutrients in abundance, resulting in excess nutrients not being absorbed by the plants. This aligns with the statement by Francis, et al. (2023) that plants in the vegetative phase have limitations in nutrient absorption from the soil.

At two WAP, the treatment of nutrient solution at a dose of 600 mL had a significantly different effect, but there was no significant difference compared to the 400 mL dose treatment. The best treatment for increasing the number of leaves in the plants was found at a dose of 200 mL, although this increase was not statistically significant. The lack of significant effect from the nutrient solution doses on the number of leaves at four and six WAP is suspected to be due to Inceptisols soil's inability to retain excess nutrients from the applied nutrient solution doses, which aligns with the statement by Ginting, et al. (2024) that Inceptisols have a low capacity to retain nutrients. Various nutrient components in the nutrient solution, such as nitrogen, phosphorus, potassium, magnesium, and others, can enhance the number of leaves in chili plants. The provision of nutrients during the vegetative phase, especially nitrogen, is capable of promoting plant growth and development, particularly in leaf formation (Rutkowski and Łysiak, 2023).

Table 4. The effect of nutrient solutions on plant height and stem diameter.

Treatments	Plant height (cm)			Stem diameter (cm)		
	2 WAP	4 WAP	6 WAP	2 WAP	4 WAP	6 WAP
Nutrient Solution Concentrations						
1200 ppm	15.74	25.85	47.39	2.09	4.34	5.76
1600 ppm	15.75	27.54	53.25	1.90	4.70	6.64
2000 ppm	15.38	27.54	51.39	2.06	4.68	6.37
Nutrient Solution Doses						
200 mL	15,42	27.70	54.72	1.98	4.59	6.65
400 mL	16,08	26.64	47.58	2.12	4.60	5.77
600 mL	15,37	26.59	49.72	1.95	4.52	6.34

Based on the results of the analysis of variance at a significance level of 5%, there was no interaction between the concentration and dose treatments of the nutrient solution on plant height and stem diameter in chili plants. This is suspected to be due to the nutrient solution suppressing the population of phosphate-solubilizing bacteria in the soil, thereby preventing the maximization of phosphorus availability in the soil (Pan and Cai, 2023). The concentration and dose treatments of the nutrient solution did not have a significant impact on plant height and stem diameter in chili plants.

The concentration of the nutrient solution at 1.600 ppm resulted in the highest plant height and stem diameter in chili plants at six WAP, although there was no significant difference compared to other nutrient solution concentrations. The lack of significant effect from the nutrient solution concentration on plant height and stem diameter is suspected to be due to poor physical, chemical, and biological properties of the soil, which hinder optimal plant development. Ezeokoli, et al. (2023) state that the addition of organic matter to the soil can improve soil health and quality, as well as increase the population of beneficial microorganisms in the soil.

Based on Table 7, the dose of nutrient solution at 200 mL achieved the highest results for plant height and stem diameter at six WAP, although there was no significant difference compared to other nutrient solution doses. The lack of significant effect from the nutrient solution doses on plant height and stem diameter in chili plants is suspected to be due to the plants not effectively absorbing macronutrients, resulting in suboptimal growth in height and stem diameter. Nitrogen, as an essential nutrient, plays a crucial role in plant growth during the vegetative phase (Leghari et al., 2016). Potassium promotes cell enlargement and elongation, which contributes to greater stem girth and height in plants (Bulawa et al.., 2022)

Yield of Chili Plants (Number and Weight of Fruits each Plant). Based on the analysis of variance at a significance level of 5%, there is an interaction between the concentration and dosage treatments of the nutrient solution on the number of chili fruits each plant.

The nutrient solution with a concentration of 2.000 ppm and a dosage of 600 mL produced the highest number of fruits per chili plant compared to other treatments, with an average of 44.67 fruits each plant (Table 5). The increase in nutrient solution concentration at 2000 ppm at each dosage level raised the average number of fruits per plant. Similarly, increasing the dosage of the nutrient solution to 600 mL at each concentration level also enhanced the average number of fruits per plant.

Table 5. The effect of nutrient solutions on number of chili fruits each plant.

Nutrient Solution Concentrates	Nutrient Solution Doses		
	200 mL	400 mL	600 mL
1200 ppm	13.67 a A	21.67 a A	18.33 a A
1600 ppm	18.00 a A	22.33 a A	27.67 a A
2000 ppm	23.33 a A	26.00 a A	44.67 b B

Notes: average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 0.05. Lowercase letters are read vertically, comparing the three concentrations at the same dosage, while uppercase letters are read horizontally, comparing the three dosages at the same concentration.

Table 6. The effect of nutrient solutions on weight of chili fruits each plant.

Nutrient Solution Concentrations	Nutrient Solution Doses		
	200 mL	400 mL	600 mL
1200 ppm	343.33 a A	449.33 a A	510.67 a A
1600 ppm	531.67 a A	501.00 a A	308.00 a A
2000 ppm	366.33 a A	405.33 a A	725.53 b B

Notes: average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 0.05. Lowercase letters are read vertically, comparing the three concentrations at the same dosage, while uppercase letters are read horizontally, comparing the three dosages at the same concentration.

The application of nutrient solution concentrations and dosages that provide various macro and micronutrients is believed to assist plants in the fruit formation process. Phosphorus plays a role in cell division and photosynthesis, while potassium helps distribute energy from the leaves to the fruits. Adequate availability of these two nutrients is expected to increase both the number and weight of fruits on the plants Kakar et al., 2024).

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Based on the analysis of variance at a significance level of 5%, there is an interaction between the concentration and dosage treatments of the nutrient solution on the weight of chili fruits each plant.

The treatment with a concentration of 2000 ppm and a dosage of 600 mL produced the highest fruit weight per chili plant compared to other treatments, amounting to 725.53 g per plant (Table 6). The increase in nutrient solution concentration at 2000 ppm at each dosage level raised the average fruit weight per plant. Similarly, increasing the dosage of the nutrient solution to 600 mL at each concentration level also enhanced the average fruit weight per plant.

As with the number of fruits, the amount of nutrient solution applied is directly proportional to the weight of the fruits per plant. The fulfillment of nitrogen nutrients through the concentration and dosage treatments of the nutrient solution is believed to help increase the number of leaves on the plants, allowing them to capture more energy for photosynthesis. Phosphorus and potassium contribute to root growth, enzyme activity, and photosynthesis, and their existence is expected to enhance both the weight and number of fruits produced by the plants (Akram et al., 2017).

Yield Quality (*Grading*). Based on the results of the variance analysis at a significance level of 5%, there is an interaction between the treatment of concentration and dosage of nutrient solution on the quality of grade A chili yields.

Table 7. The effect of nutrient solutions on quality of grade A chili yields.

Nutrient Solution Concentrations	Nutrient Solution Doses 200 mL	400 mL	600 mL
1200 ppm	5.00 a A	8.67 a A	8.67 a A
1600 ppm	9.00 a A	12.67 a A	8.67 a A
2000 ppm	10.67 a A	13.33 a A	24.33 b B

Notes: average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 0.05. Lowercase letters are read vertically, comparing the three concentrations at the same dosage, while uppercase letters are read horizontally, comparing the three dosages at the same concentration.

Based on Table 7, the interaction of a concentration of 2.000 ppm and a dosage of 600 mL of nutrient solution significantly increases the yield of grade A chili peppers. The treatment with a concentration of 2.000 ppm and a dosage of 600 mL is the best treatment for producing the highest number of grade A fruits, with an average yield of 24.33 fruits. The fulfillment of both macro and micronutrients is believed to enhance the production yield of chili plants, in line with the research by Ahmed, et al. (2024), which states that micronutrients are essential for the promotion of plant growth and the optimization of yields in horticultural systems, thus potentially improving the fruit grade as well. The total number of grade A fruits is 303, which is more than those with grade B and C.

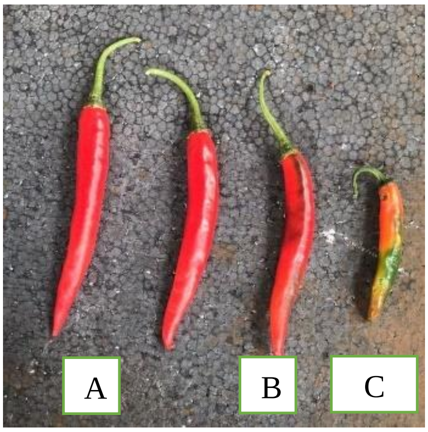

Figure 1. Chili fruit grades.

Phosphorus (P) plays a significant role in root development, flowering, and fruit ripening, which means that an increase in the number of flowers can enhance the potential yield of fruits in plants. Akram, et al. (2017) stated that adequate fulfillment of P and potassium (K) in plants can improve flower growth, positively affecting both the quantity and weight of chili fruits. The availability of nitrogen and potassium that can be absorbed by plants can yield good results, with nitrogen promoting healthy vegetative growth, particularly in stems and leaves, while potassium maintains the plant's metabolism effectively.

Based on the results of the variance analysis at a significance level of 5%, there is no interaction between the concentration and dosage treatments of the nutrient solution on the yield of grade B chili yields. This is suspected to be because the nutrients from the nutrient solution have been directed towards producing better fruit grade (grade A). The independent effects of concentration and dosage in the nutrient solution treatment significantly influence the quality of grade B chili pepper yields.

Table 8. The effect of nutrient solutions on the quality of grade B chili yields.

Treatments	Yield Quality (Grade B)
Nutrient Solution Concentrations	
1200 ppm	7.33 a
1600 ppm	8.89 ab
2000 ppm	11.89 b
Nutrient Solution Doses	
200 mL	6.78 a
400 mL	10.33 b
600 mL	11.00 b

Note: Numbers marked with the same letter indicate no significant difference based on the Duncan's Multiple Range Test at a significance level of 5%.

Based on Table 8, the treatment with a concentration of 2000 ppm of the nutrient solution significantly increases the yield of grade B chili peppers, although it does not differ significantly from the concentration of 1600 ppm. Table 10 shows that there is an independent interaction from the nutrient solution concentration treatment. As the concentration of the nutrient solution increases, the number of grade B chili peppers produced also increases. The best concentration for producing the highest number of grade B fruits is 2000 ppm, with an average yield of 11.89 grade B fruits however, this does not differ significantly from the concentration of 1.600 ppm, which has an average yield of 8,89 grade B fruits. The suspected increase in grade B chili yields due to the concentration of the nutrient solution is attributed to the fulfillment of nutritional needs during the vegetative phase, which can enhance root expansion for more optimal nutrient absorption. This aligns with research conducted by Takahashi and Katoh (2022), which indicates that a wider root distribution allows plants to reach a larger area, making nutrient absorption more efficient.

As the dosage of the nutrient solution increases, the number of grade B chili peppers produced also increases. The best dosage for producing the highest number of grade B fruits is 600 mL, with an average yield of 11.00 grade B fruits however, this does not differ significantly from the dosage of 400 mL, which has an average yield of 10,33 grade B fruits. The increase in grade B chili yields is directly proportional to the increase in the dosage of the nutrient solution, which is believed to be due to the fulfillment of the plant's nutritional needs. This is in line with research by Akram (2017), which states that abundant availability of phosphorus and potassium can enhance the weight of plant fruits.

Based on the results of the variance analysis at a significance level of 5%, it shows that there is no interaction between the concentration and dosage treatments of the nutrient solution on the quality of grade C chili yields. This is suspected to be due to pest and disease attacks that damage the physiological condition of the fruits, leading to a degradation in fruit quality. The independent effects of applying concentration and dosage of the nutrient solution do not significantly influence the quality of grade C plant yields.

Table 9. The quality of grade C chili yields.

Treatments	Yield Quality (Grade C)
Nutrient Solution Concentrations	
1200 ppm	3.11
1600 ppm	3.67
2000 ppm	3.33
Nutrient Solution Doses	
200 mL	3.33
400 mL	3.22
600 mL	3.55

The suspected cause of damage to the fruits, which results in them being classified as grade C, is plant pests. The attack from these pests can reduce the fruit's weight and create physical defects. The fruit fly is one of the pests that can attack chili fruits by puncturing them to lay their eggs. When the eggs hatch, the larvae feed on the fruit, causing it to lose weight, rot quickly, and eventually drop off. In addition to fruit flies, a common disease affecting chili plants is anthracnose. This disease is caused by the fungus Colletotrichum sp., and symptoms include small, round, dark brownish spots that are watery on the fruit. If these spots expand, they can lead to rot.

Conclusion

The concentration and dosage of the nutrient solution significantly affect the number of fruits each plant, fruit weight each plant, and the quality of grade A chili yields. However, they do not significantly influence phosphorus availability, population of phosphate-solubilizing bacteria, dry weight of the plants, phosphorus uptake, number of leaves, plant height, stem diameter, or the quality of grade B

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and C chili yields. The independent effects of nutrient solution concentration significantly impact the population of phosphate-solubilizing bacteria, dry weight of the plants, phosphorus uptake, and grade B chili yields. The independent effects of nutrient solution dosage significantly influence the growth in the number of leaves and grade B chili yields. A concentration and dosage of 2000 ppm + 600 mL can be an alternative fertilization method for cultivating red chili plants in soil media in polybags. Further research is needed regarding soil health indices such as pH and organic carbon.

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Impacts of gibberellin application on citrus: an overview from seed germination to fruit quality rnhancement

Abstract. Gibberellins are key plant hormones, and their application in citrus plants is frequently reported. This review aims to provide an overview of the application of gibberellic acid (GA3) in citrus cultivation, emphasizing their effects on growth, flowering, fruit quality, and ripening. Citrus fruits often face challenges like irregular fruit set, size inconsistency, and environmental stress, impacting yield and profitability, with exogenous gibberellin application as a potential solution. Applying gibberellins can accelerate citrus seed germination and seedling growth, enhance vegetative growth, inhibit flowering, delay fruit ripening, and improve fruit quality. However, the efficacy of gibberellins varies across species and conditions, highlighting the need for reference studies. This work presents an alternative option for optimizing gibberellin use to support sustainable citrus production practices.

Keywords: Exogenous Gibberellin · Flowering · Fruit Ripening · Fruit Quality · Seed Germination.

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Introduction

Gibberellins, a class of plant hormones, have long been recognized for their pivotal role in regulating a wide range of plant physiological processes. Since their discovery, these hormones have been widely studied and used in agriculture to boost plant growth, development, and yield. Citrus trees have become a major focus of research on gibberellins because of their economic value and the challenges involved in growing them (Gill et al., 2022).

Citrus fruits, including oranges (*Citrus sinensis*), lemons (*C. limon*), grapefruits (*C. paradisi*), and mandarins (*C. reticulate*), are among the most widely grown and consumed fruits globally (Spreen et al., 2020; Richa et al., 2023). These fruits are not only valued for their nutritional content and refreshing flavor but also represent a critical component of the agricultural economy in many regions (Vashisth & Kadyampakeni, 2020). However, citrus cultivation is often challenged by issues such as irregular fruit set (Ahmad et al., 2022), size variability (Singh et al., 2022), alternate bearing cycles (Raveh et al., 2020; Goldschmidt & Sadka, 2021; Hazarika, 2023), and susceptibility to environmental stressors (Bacelar et al., 2024). These challenges can significantly affect fruit quality, marketable yield, and, ultimately, the profitability of citrus farming.

In response to these challenges, the application of gibberellins in citrus cultivation has gained considerable attention from researchers and growers. Gibberellins are known to influence several key aspects of plant growth and development, including cell elongation, seed germination, flowering, and fruit development (Miransari & Smith, 2014; Hedden & Sponsel, 2015; Rahman et al., 2018; Hedden & Thomas, 2016; Vishal & Kumar, 2018; Binenbaum et al., 2018). Gibberellin has shown the potential to address some of the most pressing issues in citrus cultivation by promoting more uniform fruit development (Livingston & Vashisth, 2022), synchronizing flowering (Bauerle, 2022), enhancing fruit size (Elmenofy et al., 2021), and improving overall yield (Kumara et al., 2023).

This review aims to provide a comprehensive overview of the current knowledge on gibberellin application in citrus, exploring its effects on growth, yield, and quality parameters. By exploring the existing literature, this review seeks to highlight the potential benefits and limitations of gibberellin use in citrus cultivation, offering insights for researchers and growers looking to optimize citrus production. Through a detailed examination of gibberellin's impact on citrus, this review contributes to the ongoing efforts to improve agricultural practices and enhance the economic viability of citrus farming.

Gibberellin accelerates seed germination and seedling growth. Gibberellins can significantly enhance the germination process by breaking seed dormancy (Lee et al., 2022), promoting the mobilization of stored nutrients (Niharika et al., 2021; Xiong et al., 2022), and accelerating the radicle emergence (Wu et al., 2021; Ge et al., 2023).

Table 1. Impact of gibberellin on seed germination and seedling growth of citrus.

Citrus Species	Gibberellin Concentration	Application Time	Effect	Reference
Kagzi Lime	80 mg/L*	12 hours	Increase germination percentage and growth	Dilip et al., 2017
Acid Lime	3000 mg/L	Seed soaking overnight	Maximum growth in respect of germination percentage, height of seedling, number of leaves, stem diameter, and leaf area	Meshram et al. (2015); (Al-Musawi et al., 2020)
Cleopatra mandarin	2000 mg/L*	Seed soaked 24 hours	Increase germination percentage, leaf mineral content and total carbohydrate content	Sharaf et al. (2016); El-Sayed (2018)
Pummelo	50 mg/L*	Seed soaked 24 hours	Accelerate germination and growth	Khopkar et al., 2017
Rangpur lime	750 mg/L	16 hours of light at 30 °C and 8 hours of darkness at 20 °C	Accelerate germination, form vigorous seedlings and increase SOD and POD activity	Neto et al., 2024

*: converted from ppm

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This results in a more uniform and faster germination rate compared to untreated seeds. Additionally, gibberellin application can influence seedling growth by stimulating cell elongation and division (Shah et al., 2023), leading to taller and more vigorous seedlings with enhanced root and shoot development. The overall impact of gibberellins on citrus seed germination and seedling growth is highly beneficial, contributing to improved early-stage plant establishment and potentially higher yields (Adhikary, 2022).

Germination and seedling growth parameters were enhanced by soaking seeds from various plants in GA3 and Zn. Studies by Meshram et al. (2015) on acid lime, Sharaf et al. (2016) on Cleopatra mandarin and Rangpur lime, Dilip et al. (2017) on Kagzi lime and Rangpur lime, Khopkar et al. (2017) on Pummelo, and El-Sayed (2018) on Cleopatra mandarin have demonstrated these effects. The germination percentages were significantly increased following by GA3 treatments. However, higher concentrations of GA3 and $ZnSO_4$ (750, 1500, and 3000 mg/L) led to a reduction of seed germination percentage of acid lime, while promoting better vegetative growth (Al-Musawi et al., 2020).

Gibberellic acid (GA) at 80 ppm has been shown to significantly affect seed germination and seedling growth in Rangpur lime, making it a recommended treatment for growers seeking enhanced growth and yield (Dilip et al., 2017). The application of GA3 significantly accelerates the germination process and promotes improved seedling growth and development, resulting in more vigorous seedlings. Furthermore, GA3 treatment not only accelerates germination but also increases enzymatic activities, such as superoxide dismutase (SOD) and peroxidase (POD), which help mitigate oxidative stress and enhance seedling vigor in Rangpur lime cv. Santa Cruz (Neto et al., 2024). Gibberellin increases the activities of antioxidant enzymes like SOD and POD because it activates protective mechanisms against oxidative stress (Forghani et al., 2020; Zhu et al., 2019). Gibberellins are involved in the plant's response to stress, including oxidative stress, which occurs when there is an accumulation of ROS such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) (Mishra et al., 2017). Both SOD and POD are antioxidant enzymes that help neutralize these harmful ROS and protect plant cells from oxidative damage. Accordingly, Table 1 summarizes several studies on the effects of various gibberellin concentrations on seed germination and seedling growth in citrus.

Gibberellin enhances vegetative growth. By stimulating cell division and elongation, gibberellin can increase shoot length, leaf area, and overall plant vigor. This enhanced vegetative growth is beneficial for citrus trees, as it can lead to increased yield potential and improved fruit quality. Moreover, gibberellin can influence the branching pattern of citrus trees (Castro-Camba, 2022). By promoting the development of lateral shoots, gibberellin helps create a more open and productive canopy (Ni et al., 2015), which facilitates better light penetration (Huber et al., 2021) and enhances fruit set. While gibberellin application can be a valuable tool for improving citrus vegetative growth, it is important to note that the optimal concentration and timing of application may vary depending on the citrus variety, environmental conditions, and specific growth objectives.

Table 2. Impact of various applications of gibberellin on vegetative growth of citrus.

Citrus Species	Gibberellin Concentration	Application Time	Effect	Reference
Kaffir lime	200 mg/L	Once a week until 8 weeks	Longest flush, increase in the number and weight of leaves	Budiarto et al., 2023
Acid lime cv. Hasta Bahar	50 mg/L	Once in 7-10 days interval	Increase in plant height, canopy spreads, canopy volume, and shoot length	Rai et al., 2018
Acid lime	3000 mg/L	-	Increase plant height, stem diameter, leaf number, leaf area, plant fresh and dry weight	Al-Musawi et al., 2020
Acid lime cv. Vikram	50 mg/L	-	Increase height, number of leaves, and girth of stem	Parmar et al., 2019

Without pruning, the application of 0.02% gibberellin could produce the longest flush, with increases in the number and weight of leaves by about 77% and 64%, respectively, compared with the control (Budiarto et al., 2023). Gibberellin application also significantly influenced the number of shoots. Plant height, stem diameter, leaf number, leaf area, and both fresh and dry plant weight were progressively increased with higher concentrations of GA3 (Al-Musawi et al., 2020). GA3 application at 50 ppm recorded the highest increase in acid lime height, canopy spreads, canopy volume, and shoot length (Rai et al., 2018). Table 2 summarizes various studies that examine the effects of different gibberellin concentrations on vegetative growth of several citrus species.

Gibberellin inhibits flowering. Earlier study indicates that gibberellin application before flowering can counteract the floral induction in citrus (Garmendia et al., 2019). In citrus trees, gibberellin application can significantly influence the timing and intensity of flowering (Agustí et al., 2022). These hormones are commonly used to manipulate the flowering process, particularly in commercial citrus production, to enhance yield and synchronize fruit development. Gibberellins promote floral bud growth, break dormancy, and encourage early flowering (Thirugnanasambantham et al., 2020). However, the effects of gibberellin application can vary depending on the timing, concentration, and environmental conditions. When applied appropriately, gibberellins can result in more uniform flowering, which is beneficial for the overall productivity and quality of citrus crops. Conversely, improper application can lead to uneven flowering or excessive vegetative growth at the expense of flower production, ultimately affecting fruit yield. Therefore, understanding the optimal conditions and proper use of gibberellins is crucial for maximizing their benefits in citrus cultivation.

Exogenous GA applications during the induction period consistently reduce flower formation (Kupke et al., 2022). The use of 40 mg/L GA3 in two, three, or four sequential applications, from May to June, May to July, and from May to August, at 21-day intervals, reduces the intensity of flowering and sprouting in alternate-bearing plants during the subsequent spring (Griebeler et al., 2021). This treatment increases the number of mixed shoots while decreasing the number of floral shoots. Gibberellins increase the number of mixed shoots by promoting vegetative growth and cell elongation. At the same time, they decrease the number of floral shoots because they inhibit the transition from vegetative growth to flowering due to the repression of the expression of the Citrus FLOWERING LOCUS T (CiFT) gene (Bennici et al., 2021). Flowering in citrus is inhibited by the application of gibberellins before inductive stress conditions. As a result, antigibberellin chemical compounds, such as CCC, SADH, benzothiazole, and triazoles have been observed to induce flowering in citrus (Huchche & Ladaniya, 2014). The application of GA3 at 21 mg/L reduced the formation of floral structures, leading to a quadratic increase in field production by reducing competition for assimilates among fruits in 'Tahiti' acid lime (Pereira et al., 2014).

Gibberellin delays fruit ripening. It is important to highlight the role of gibberellic acid in delaying fruit aging. GA treatments can reduce or correct peel disorders, such as blemishes and pitting, by creating a denser albedo texture when applied to citrus fruits still on the tree (Kalatippi, 2024). During the regreening process, fruits treated with GA turned green more rapidly. Compared to untreated fruits, GA treatment induces chlorophyll accumulation and decreases the content of carotenoids, such as β-cryptoxanthin, all-*trans*-violaxanthin, and 9-*cis*-violaxanthin, in Valencia oranges (Keawmanee et al., 2022).

Gibberellic acid delays peel senescence. In addition to its favorable effects on peel disorders, GA causes a typically undesirable delay in degreening, whether applied on the tree or after harvest (Lurie, 2024; Rezk et al., 2024). However, this effect has proven beneficial for lemons in cooler regions of California, where they mature later in the winter, as well as for grapefruits in semi-tropical areas (Aliyev & Latif, 2022; Adewoyin et al., 2023). Additionally, dipping harvested fruits in GA solutions helps delay the formation of pigments associated with fruit aging and preserves healthy peel tissues, a technique now being adopted on a commercial scale.

Pre-harvest application of GA3 has been shown to delay fruit softening (Ayaz et al., 2023), slow down rind color change (Yamaga et al., 2024), and reduce fruit drop and puffiness (Khalil, 2020). Gibberellins can extend the period

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Table 3. Impact of various applications of gibberellin on delay fruit ripening of citrus.

Citrus Species	Gibberellin Concentration	Application Time	Effect	Sources
Mandarin orange	30 mg/L*	-	Extending shelf life and reducing physiological weight loss	Rokaya et al., 2016
Valencia orange	0.5 mg/L	Every 2 weeks for 3 times	Delays peel color development and increases PPF	Keawmanee et al., 2022
Kumquat	20 mg/L	-	Lower contents of abscisic acid and titratable acid	Cai et al., 2021

before ripening starts by slowing down the processes that lead to the breakdown of cellular structures and the loss of chlorophyll (Baseer et al., 2024). Ethylene is a key hormone for initiating ripening, gibberellins can interact with ethylene signaling to modify the timing or intensity of ripening (Alferez et al., 2021). Gibberellins can effectively enhance fruit storage quality by significantly increasing flesh firmness, reducing respiration rate, inhibiting the release of endogenous ethylene, and preventing fruit softening and ripening (Zhang et al., 2023). Additionally, GA3 improves both the internal and external quality of stored fruit by enhancing fruit shape, regulating color, delaying the decline of soluble solids, promoting sugar accumulation, and slowing vitamin loss. A gibberellin application at 20 ppm was particularly effective in increasing fruit weight and overall quality, while a concentration of 30 ppm was superior for extending shelf life and reducing physiological weight loss and decay in mandarin oranges (Rokaya et al., 2016). Pre-harvest application of GA3 spray at a concentration of 20 mg/L had a positive effect on the nutritional quality and exterior color of 'Jindan' kumquat fruits (Cai et al., 2021). The results also indicated that GA3 treatment significantly increased the contents of IAA, GA, ZRs, TSS, and VC in kumquat. Table 3 summarizes several studies on the effects of applying various concentrations of gibberellin on delay fruit ripening of citrus.

Gibberellin improves fruit quality. The application of gibberellins, particularly GA3, has been extensively studied for its effects on citrus fruiting. When applied to citrus trees, gibberellins can significantly influence fruit set, size, and quality. For example, GA3 application is known to enhance fruit set by reducing fruit drop during the early stages of development. It also promotes fruit enlargement by stimulating cell division and elongation, leading to larger and more uniform fruits. Additionally, gibberellins can delay fruit maturation, which is beneficial for extending the harvest period and improving fruit quality by allowing more time for sugar accumulation. However, the effects of gibberellin application can vary depending on the timing, concentration, and method of application, as well as the specific citrus variety being treated. Proper management of gibberellin application is crucial for maximizing its benefits and avoiding potential negative effects, such as excessive vegetative growth at the expense of fruit development.

Gibberellic acid at 50 ppm increased the level of Chlorophyll (Ch) a, Ch b, Ch total (Ch a+b), carotenoid content, fruit juice content, vitamin C, as well as the activities of superoxide dismutase (SOD) and peroxidase dismutase (POD). Meanwhile, the highest fruit firmness and total soluble solids (TSS) were recorded with 40 ppm GA3 (Khan et al., 2024). GA_3 can increase chlorophyll content in plants by promoting cell division and expansion, leading to larger and more functional chloroplasts (Bagnazari et al., 2018). It also enhances the activity of enzymes involved in chlorophyll biosynthesis, such as glutamyl tRNA reductase and porphobilinogen deaminase, which are critical for the production of chlorophyll (Chai et al., 2023). The increase in chlorophyll content improves the plant's ability to perform photosynthesis (Croft et al., 2017). This is important for energy production and growth, especially in young plants or during periods of active growth. GA_3 also affects the carotenoid biosynthesis pathway by stimulating the expression of genes encoding enzymes involved in carotenoid production, such as phytoene synthase and lycopene β-cyclase (Keawmanee et al., 2022).

Table 4. Impact of various applications of gibberellin on fruit quality of citrus.

Citrus Species	Gibberellin Concentration	Application Time	Effect	Reference
Balady Lime	20 mg/L	-	Improve physical and chemical fruit characteristics	Gomaa, 2020
Bac Son mandarin fruit	100 mg/L	-	Reduce the number of seeds and increase the quality	Hung et al., 2023
Sweet lime	50 mg/L	5, 10 and 15 days after bud break	High values of Ch a, Ch b, Ch total, carotenoid, fruit juice, vitamin C, superoxide dismutase (SOD) and peroxidase dismutase (POD) activity	Khan et al., 2024
Kinnow Mandarin	25, 45, and 65 mg/L	At pre-harvest stage	Improved the physical and biochemical properties	Talat et al., 2020

GA_3 plays a role in fruit growth and development. It promotes cell division and elongation, leading to larger fruit size and increased juice yield (Chauhan et al., 2020). GA_3 can also influence the metabolic pathways that control the accumulation of sugars, acids, and other components in fruit Shinozaki et al., 2020; Du et al., 2024; Ochiki et al., 2024). GA_3 may influence the synthesis of vitamin C (ascorbic acid) by stimulating the enzymes involved in the biosynthetic pathway (Arya & Viji, 2024).

Spraying GA3 in combination with naphthalene acetic acid (NAA) significantly improved the physical and chemical characteristics of Balady lime fruit (*Citrus aurantifoli*a L) (Gomaa, 2020). In previous experiment, when fruit set increased excessively due to GA applications, a noticeable reduction in average fruit weight was recorded, indicating lower commercial value (Bisht et al., 2018). Spraying GA3 also resulted in a lower seed count and a higher actual yield in Bac Son mandarin fruit (Hung et al., 2023). The application of GA3 at 65 ppm significantly improved physical attributes such as peel color, fruit weight, and juice weight, while the application of GA3 at 25 ppm and 45 ppm enhanced the biochemical quality attributes (total soluble solids, acidity, ascorbic acid, antioxidants, phenols, flavonoids, and carotenoids) of Kinnow fruit (Talat et al., 2020). Table 4 summarizes various studies examining the effects of different concentrations of gibberellin on fruit quality of several citrus species.

Future Perspective. Gibberellin has shown significant promise in influencing various aspects of citrus growth and development. Future research could investigate deeper into the mechanisms by which gibberellin promotes seed germination, vegetative growth, and flowering in citrus. Additionally, exploring the potential of gibberellin inhibitors to delay fruit ripening and enhance fruit quality could offer innovative agricultural practices. Understanding the complex interactions between gibberellin and citrus physiology could allow researchers to develop targeted strategies to optimize fruit production and quality, ensuring a sustainable citrus supply for consumers worldwide.

Further studies might also focus on the effects of combining gibberellin with other plant growth regulators to identify synergistic effects that optimize growth stages, improve stress tolerance, and enhance nutrient uptake. This research could reveal methods to balance gibberellin with hormones such as auxin and cytokinin, potentially leading to proper management of plant physiology in response to environmental changes. Ultimately, continued exploration of gibberellin's multifaceted role in citrus development may result in the establishment of more adaptable, resource-efficient, and high-yielding orchards, supporting the global citrus supply and reducing the environmental footprint of citrus production.

Conclusion

Gibberellins have been extensively studied for their potential to improve various aspects of citrus production. Gibberellin applications can positively influence fruit sets, delay ripening, and enhance fruit quality, contributing to better marketability; however, the impacts may vary depending on application timing, concentration, and cultivar. Those variabilities highlight the need for tailored gibberellin application strategies. This overview compiles data to maximize gibberellin benefits for sustainable citrus production.

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An overview of gibberellin inhibitors for regulating vegetable growth and development

Abstract. Gibberellin (GAs) promotes plant growth processes like germination, shoot elongation, root development, and flowering, while its inhibition is occasionally used in some vegetable crop practices. This paper aims to review the current literature on GAs inhibitors in practical vegetable crops and suggest future strategies for increasing yield. In some vegetable crops, inhibiting GAs with natural (abscisic acid, ethylene) or synthetic (paclobutrazol, prohexadione-Ca) regulators is key to improving yield and quality. Abscisic acid (ABA) counteracts GAs in germination and stress adaptation, while ethylene (ET) opposes GAs in senescence and growth inhibition. The application of paclobutrazol (PBZ) and prohexadione-Ca (Pro-Ca) is known to inhibit synthetic GAs, resulting in denser plants while improving plant photosynthetic efficiency, which improves crop yield. Achieving desired growth and yields with GAs inhibitors relies on precise dosing, emphasizing the importance of review studies for advancing vegetable cultivation.

Keywords: Abscisic acid · Ethylene · Paclobutrazol · Plant growth · Prohexadione-Ca.

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Introduction

Plant growth is a complex biological phenomenon regulated by numerous factors, among which plant growth regulators (PGRs) play a vital role. PGRs are both naturally and synthetically occurring low-molecular-weight compounds that, even at minimal concentrations, regulate a range of physiological processes and developmental stages within plants (Karabulut *et al.*, 2024), such as plant canopy growth, root development, fruit formation, fruit, and leaf abscission (Fenn & Giovannoni, 2021). PGRs also modulate plant responses to abiotic stress, aiding in minimizing damage from external stress factors (Assaf *et al.*, 2022). Among plant hormones, gibberellin, auxin, and cytokinin are popularly known as growth-promoting, while abscisic acid (ABA) and ethylene (ET) are recognized for their growth-inhibiting functions.

Gibberellins (GAs) are central to plant growth and development. They help with processes such as cell division, stem elongation, seed germination, dormancy, leaf expansion, and the development of flowers and fruits (Ahmad *et al.*, 2018). Of the over 130 GAs found in plants, fungi, and bacteria, only GA1, GA3, GA4, and GA7 are believed to function as bioactive hormones (Hedden & Sponsel, 2015). GAs play a key role in regulating shoot elongation in carrots (Wang *et al.*, 2017) and have emerged as crucial regulators of root meristem development (Shtin *et al.*, 2022). At the molecular level, GAs promote plant elongation by regulating cell growth. In tomatoes, a gene linked to dwarfism is associated with the GAs metabolic pathway and encodes an enzyme involved in this process (Sun *et al.*, 2019).

GAs interact with other plant hormones to coordinate growth responses, often balancing with growth-inhibiting hormones like ABA and ET. ABA is a crucial phytohormone that plays a key role in plant development and stress adaptation, making it essential for enhancing plant resilience under varying environmental conditions (Malik *et al.*, 2023). Its influence extends to processes such as leaf senescence, vegetative-reproductive phase transitions, seed dormancy, and germination, all of which are important for managing the plant life cycle (Ali *et al.*, 2022). Additionally, (ET) is a versatile phytohormone that can either promote or inhibit plant growth and senescence, depending on factors such as concentration, timing, and specific plant species (Iqbal *et al.*, 2017). Moreover, growth retardants or inhibitors, such as paclobutrazol (PBZ), and prohexadione-Ca (Pro-Ca), are externally applied to regulate GAs activity. In certain crops, GAs inhibitors are used to boost yields, e.g., potato (Hamdani *et al.*, 2019), cucumber (Başak, 2021), mango (Sarker *et al.*, 2016), mandarin citrus (Darmawan *et al.* 2014) and tomato (Altuntaș, 2016). Growth inhibitors are particularly valuable in managing GAs activity, allowing the plant to direct its energy toward targeted organs rather than excessive vegetative growth, such as reported in potato tuber (Nuraini *et al.*, 2016).

An in-depth overview of GAs inhibitors' applications and their potential in vegetable crop cultivation is essential. As the demand for sustainable and efficient vegetable production grows, understanding the current condition of GAs inhibitor research will support optimized crop management and yield improvements. This review aims to synthesize existing literature on GAs inhibitors and their role in practical agriculture and outline future directions for enhancing yield.

ABA and ET regulate seed germination performance. ABA is a key hormone involved in regulating seed dormancy, maturation, and germination, playing a crucial role in determining the timing of these processes (Nonogaki, 2017). Its interactions with GAs, which oppose each other, are also essential in deciding whether a seed stays dormant, germinates, or develops into a seedling (Shu *et al.*, 2018; Hoang *et al.*, 2014; Lee *et al.*, 2015a; Kong *et al.* 2015). The study by Groot and Karssen (1992) showed that 50% inhibition of germination in Lycopersicon esculentum seeds occurs with exogenous ABA at concentrations of approximately 0.53 mg/L and 1.32 mg/L (equivalent to 2 μM and 5 μM, respectively). As in Arabidopsis, the ABA fraction in the tomato embryo and endosperm is essential for dormancy induction (Groot and Karssen, 1992). This experiment confirms that ABA plays a crucial role in inducing dormancy during seed development in tomatoes. During seed maturation, the ABA level in the seed increases, helping to maintain seed dormancy and prevent vivipary (Shu *et al.* 2016). ABA is transported from the endosperm to the embryo to inhibit its growth (Kang *et al.*, 2015). Towards germination, the ABA level begins to decrease and the GAs content increases after imbibition and stratification treatments (Shu *et al.*, 2016).

Table 1. Effect of exogenous ET application on vegetable seed germination.

Species	Concentration	Method	Effect	References
Allium cepa	600 mg/L*	Spray at 45 DAP	Increase 19% of germination and seed vigor than the control	Yalamalle *et al.* 2020
		Spray at 60 DAP	Increase 51.65% of germination and seed vigor than the control	
Brassica oleracea	150 mg/L**	Placed on filter paper moistened with NO for 5 days	NO promote ethylene production and break secondary dormant of *Brassica oleracea*	Sami *et al.* (2019)
Amaranthus retroflexus	20.62 mg/L**	Placing seeds after long-term storage on filter paper moistened with ethephon solution for 7 days at 25 °C and 35 °C	Enhanced seed germination up to 70-85% after long-term storage	Kępczyński & Sznigir (2014)

* : convert from ppm; ** : convert from M; DAP: day after planting.

After seed shedding, dormancy is released through ABA degradation during imbibition, which then triggers germination through the action of GAs (Sano & Marion-Poll, 2021). The balance between these two hormones regulates light, temperature, and nitrate signals, acting oppositely to control embryo growth and endosperm softening (Carrera-Castaño *et al.*, 2020; Chahtane *et al.*, 2017; Tuan *et al.*, 2018). In this context, An and Zhou (2017) demonstrate that light induces germination in Lactuca sativa by promoting the production of nitric oxide (NO), a reactive nitrogen molecule that counteracts ABA during seed germination (Albertos *et al.*, 2015). Furthermore, NO is crucial for the transcription of GA3ox1 and GA3ox2, two key enzymes involved in the biosynthesis of active GAs in Arabidopsis (Bethke *et al.*, 2007). Recent studies have expanded the understanding of ABA's role, highlighting its significant contributions to seed adaptive responses against both abiotic and biotic stresses, beyond just dormancy (Sano & Marion-Poll, 2021; Pan *et al.*, 2021).

ET is one of the plant hormones that help regulate seed dormancy and germination in many species (Santos & Garcia, 2023). ET production is generally low during phase 1 (imbibition) and phase 2 (germination) but increases during phase 3 (growth) when the root elongates through the seed coat (El-Maarouf-Bouteau *et al.*, 2015). Radicle protrusion is always associated with a peak in ET production .ET stimulates seed germination in various species. It is known that exogenous ET can break the dormancy of *Brassica oleracea* and *Lactuca sativa* (Corbineau, 2024; Sami *et al.*,2019). Exogenous ET can increase germination and seed vigor of *Allium cepa* (Yalamalle *et al.*, 2020). The effect of exogenous ET application on seed dormancy and germination in various vegetable species is shown in Table 1.

Anti-gibberellin regulates vegetative growth . ABA is a hormone that inhibits plant growth. High concentrations of exogenous ABA can halt growth, while endogenous ABA accumulates during stress, reducing growth. ABA signaling directs the plant to focus on survival rather than growth (Brookbank *et al.*, 2021). However, the effects of ABA on growth can vary depending on concentration, timing, and the plant part involved (Humplík *et al.*, 2017; Mabvongwe *et al.* 2016). ABA-mediated growth regulation crosstalk with other hormones, particularly ET (Khan *et al.*, 2024), to control various cell growth processes. Similar to natural phytohormone, synthetic anti-gibberellin such as PBZ and Pro-Ca is frequently reported to modify plant growth by decreasing the dominance of vegetative growth (Cline, 2017; Diwan *et al.* 2022), such as decreasing plant height (Lee *et al.*, 2015b). Reducing vegetative growth makes plants more compact, saving space and improving resource efficiency, especially in high-density cropping and controlled environments with limited resources (Ito *et al.*,

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2016). Similarly, plants naturally reduce vegetative growth to manage limited resources by redirecting them to the regeneration phase, ensuring survival (Tang *et al.*, 2021). It has also been found that the application of PBZ in rice reduces vegetative growth by directing more photosynthates to seed development and less to vegetative growth (Dewi *et al.*, 2016). The details of the various effects of synthetic anti-gibberellin application to regulate the vegetative growth of numerous vegetable species is described in Table 2. The reduction of vegetative growth as the impact of GAs application was also stated by numerous researchers by observing leaf development variables.

Table 2. Effects of PBZ and Pro-Ca application on the regulation of vegetative growth in numerous vegetable species

PGRs	Species	Concentration	Method	Effect	References
PBZ	*Lycopersicum esculentum*	50 mg/L	Apply treatments at 25, 77, 92, and 107 DAP	Reduce plant height and shorten internode length	Ramos-Fernández *et al.* (2020)
		250 mg/L*	Sprayed in the second week after transplanting	Reduce plant height	Novita (2022)
	Solanum melongena	200 mg/L	Apply to the root zone of seedlings using a soil injector	Increased the plant height, number of branches, number of buds	Khandaker *et al.* (2020)
	Solanum tuberosum	150 mg/L*	Spray all parts of the plant leaves	Inhibit plant height	Hamdani *et al.* (2024)
		50 mg/L	Foliar application	Suppressed plant height	Hamdani *et al.* (2019)
	Cucumis sativus	150 mg/L	Spray on the cotyledon leaves with approximately 25 shots of equal force	Reduce height by 23.93%.	Cázarez Flores *et al.* (2018)
	Capsicum frutescens	150 mg/L*	PBZ application during rainy season	Reduce height by 15.72%.	Nurrachman *et al.* (2023)
	Allium sativum	250 mg/L	Spray at 70 and 98 DAP	Increase pseudo stem diameter by 20.78%	Kristina *et al.* (2024)
Pro-Ca	*Solanum melongena*	50 mg/L	Spray at the emergence of the third true leaf (20 DAP)	Reduce shoot height by 27%	Ozbay & Ergun (2015)
		100 mg/L		Reduce shoot height by 32%	
		150 mg/L		Reduce shoot height by 38%	
	Capsicum annuum	50 mg/L	Foliar spray and soil drench	Reduce seedling (42 DAP) height by 25-31%	Özbay & Metın. (2016).
	Cucumis sativus	30 mg/L	Spray 10 and 20 DAP	Improved vegetative growth	Başak (2021).
	Solanum tuberosum	50 mg/L	40 DAP	Increased the number and weight of tubers per plant	Hernawati *et al.* (2022)
		150 mg/L*	Spray all parts of the plant leaves	Inhibit plant height	Hamdani *et al.* (2024)

* : convert from ppm; DAP: Day After Planting

Table 3. Effects of PBZ and Pro-Ca application on the leaf area reduction in numerous vegetable species.

PGRs	Species	Concentration	Method	Effect	Ref.
PBZ	*Cucumis sativus*	150 mg/L	Sprayed on the cotyledon leaves with approximately 25 shots of equal force	Suppressed leaf area by 40,12%.	Cázarez Flores *et al.* (2018)
	Solanum tuberosum	150 mg/L*	Spray all parts of the plant leaves		
	Suppressed Reduce leaf area	Hamdani *et al.* (2024)			
		50 mg/L	Foliar application	Suppressed leaf area	Hamdani *et al.* (2019)
Pro-Ca	*Solanum tuberosum*	150 mg/L*	Spray all parts of the plant leaves		
	Suppressed Reduce leaf area	Hamdani *et al.* (2024)			
	Solanum melongena	150 mg/L	Spray at the emergence of the third true leaf (20 DAP)	Suppressed leaf area of seedling (35 DAP) by 15%	Ozbay & Ergun (2015)

* : convert from ppm; DAP: Day After Planting

Table 4. Effects of PBZ and Pro-Ca application on root and tuber development of potato.

PGRs	Species	Concentration	Method	Effect	Reference
PBZ	*Solanum tuberosum*	100 mg/L*	Spray all parts of the plant leaves	Increase heavy tuber per plant	Hamdani et al. (2024)
		50 mg/L	Foliar application	Increase tuber number and percentage of class tuber for seed size	Hamdani et al. (2019)
		3 mg/L*	Pouring it into an explant bottle of in vitro	Stimulating subapical stolon and uber swelling	Pane et al. (2021)
		50 mg/L*	Spray all parts of the plant leaves	Increase tuber number per plant	Hamdani et al. (2024)
Pro-Ca		50 mg/L*			
	Solanum melongena	150 mg/L	Spray at the emergence of the third true leaf (20 DAP)	Reduce root fresh weight of seedling (35 DAP) by 12,71%	Ozbay & Ergun (2015)

* : convert from ppm

Leaf development is influenced by numerous factors, one of which is hormonal signals (Bar & Ori, 2014). ABA significantly influences leaves' senescence, with levels rising as leaves age (Chen *et al.*, 2024; Liu *et al.*, 2016). The increase in ABA content during senescence further promotes leaf aging, suggesting that ABA manipulation could regulate leaf drop (Guo *et al.*, 2021). Along with ABA, ET is also a key regulator of leaf aging and senescence, particularly in species sensitive to its effects. ET biosynthesis is elevated during early leaf development, decreases as the leaf matures, and then increases again as senescence begins (Iqbal *et al.*, 2017). Reducing the levels of functional GAs or conjugating them with glucose can lead to leaf yellowing, while exogenous GAs applications can delay senescence and reduce ET production (Iqbal *et al.*, 2017). In addition to leaf aging, leaf area variables are also important to note in vegetable cultivation (Table 3). Hence, anti-gibberellin such as Pro-Ca reduced leaf area, as well as fresh and dry leaf weights (Zhang et al, 2023), due to the block of GA synthesis that is responsible for leaf expansion (Sharma *et al.*, 2024).

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GAs inhibitor improving root and tuber development. ABA is a key plant hormone that plays a vital role in root growth (Harris, 2015; Sun *et al.*, 2018). At low concentrations, ABA promotes primary root elongation, while at high concentrations, it inhibits root growth, with both effects being regulated by auxin (Li *et al.*, 2017; Zheng *et al.*, 2022). ABA also controls the expression of genes involved in auxin production, further influencing root development (Qin *et al.*, 2017). Additionally, the application of shoot-ABA increases basipetal auxin transport by 114%, which in turn enhances root cell elongation by 56% (Xie *et al.*, 2020). Research suggests that a high ratio of ABA to GAs promotes tuber development (Chen *et al.*, 2022). In contrast, an excess of GAs can delay tuber formation. Manipulation of the dominance of ABA to GA could enhance tuber yield and development. One of the feasible strategies is the application of growth retardant such as PBZ and Pro-Ca. PBZ treatments shorten shoot length, thicken stems, and promote compact growth, enhancing root formation and overall plant structure (Desta & Amare, 2021). The details of the PBZ and Pro-Ca application for improving root and tuber production is described in Table 4

Anti-gibberellin has potential to increase flowering and accelerate fruit ripening. Flowering is a critical phase in the plant life cycle, marking the onset of conditions conducive to reproductive success (Rana *et al.* 2023). The timing of flowering is influenced by interactions between the plant and its environment, as well as by the plant's internal developmental capacity, enabling the transition from the vegetative to the reproductive phase (Cai *et al.*, 2024). Exogenous ET has been used to stimulate flowering in Bromeliads and to accelerate germination and flowering in other plants (Reid & Wu, 2018). This demonstrates the significant role of ET in flower development. PBZ and Pro-Ca have a notable impact on flowering. A 100 mg/L application of PBZ during the rainy season was found to accelerate *Capsicum frutescens* flower initiation by 6.6% (Nurrachman *et al.*, 2023). This suggests that PBZ can help speed up the flowering process under specific environmental conditions. On the other hand, a 150 mg/L application of prohexadione-Ca during the rainy season increased the yield of *Capsicum frutescens* by up to 18.3% (Nurrachman *et al.*, 2023). Concerning fruit ripening, ABA accelerates the ripening of both climacteric and non-climacteric fruits (Bai *et al.*, 2021; Kou *et al.*, 2021). In tomatoes, climacteric fruit ripening is accelerated by reducing the negative ABA signaling regulator, SlPP2C1, or by increasing the expression of the ABA receptor, SlPYL9 (Zhang *et al.*, 2018; Kai *et al.*, 2018).

Future directions. This review provides an in-depth examination of the role of anti-gibberellin PGRs and the application of synthetic anti-gibberellins in managing vegetative growth and enhancing crop productivity. Naturally occurring plant hormones, such as ABA and ET, act as endogenous GAs inhibitors, creating a balance that supports plant adaptation and controlled growth. In agriculture, synthetic anti-gibberellins like PBZ and Pro-Ca mimic these effects by directly inhibiting GAs biosynthesis, reducing excessive vegetative growth, and promoting regenerative phases that support higher yields. These synthetic inhibitors are useful and can substantially reduce production costs and improve crop quality. One critical challenge in applying anti-gibberellins is the variability in plant responses across species and cultivars, often necessitating crop-specific application protocols. Accurate dosage and application timing are crucial, as plant growth regulators are highly beneficial in agriculture when applied at both low and high concentrations (Agboola *et al.*, 2014). This review highlights the need for further research across diverse plant species to establish refined dosage and application techniques that optimize both effectiveness and safety in various cropping systems.

The environmental sustainability of PGR applications is another essential consideration. Residues from some PGRs, particularly PBZ, have shown persistence in soils, which may impact surrounding ecosystems and pose challenges for long-term agricultural sustainability. Conversely, Pro-Ca demonstrates a more rapid degradation rate, presenting a more environmentally favorable option. Addressing the environmental impact of these substances requires ongoing study and the development of innovative application techniques. One promising approach to enhance application precision is the use of nanoparticles as delivery agents for anti-gibberellin PGRs.

Nanoparticles penetrate cells efficiently, making them ideal for substance delivery. Their small size and large surface area enhance substance solubility, stability, absorption, and retention in target tissues, improving bioavailability, protecting against early degradation, and extending circulation time, with selective uptake in target cells

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(Sarker & Nahar, 2022). This precision minimizes the required dose, reduces the likelihood of chemical runoff, and ultimately contributes to a more sustainable agricultural practice. Additionally, nanoparticle-based delivery systems could improve the bioavailability of active compounds in plant cells, achieving the intended growth-regulating effects with lower environmental impact. Future research should focus on developing such precision delivery systems, assessing their effects on various crops, and conducting field trials to determine optimal conditions for application. Incorporating these advancements could pave the way for a new generation of PGR applications that not only optimize crop yield and quality but also support the global demand for environmentally sustainable agricultural practices.

Conclusion

GA positively promotes plant growth processes like shoot elongation, root expansion, and flowering. However, inhibiting GAs can also enhance yield and quality in some vegetable crops. GA inhibition is naturally caused by the dominance of ET and ABA, while synthetic GAs biosynthesis inhibitors include PBZ and Pro-Ca. ABA's antagonistic role in GA regulation is vital for processes such as seed dormancy and root development, which are particularly relevant in stress adaptation. ET opposes GAs, causing growth suppression, senescence, and sometimes greater stress resistance. PBZ, with its triazole structure, restricts GA synthesis to produce more compact plants, enhancing root development and photosynthetic efficiency—an approach particularly advantageous in intensive and controlled-environment agriculture. Optimal yields from PBZ and Pro-Ca depend on precise dosing, making studies on GAs inhibitors valuable for broader vegetable cultivation.

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