Volume 25 Issue 1, April 2026

ISSN: 1412-4718
eISSN: 2581-138x

Jurnal KULTIVASI

ORGANIZED BY : DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE, UNIVERSITAS PADJADJARAN
AND INDONESIAN AGRONOMY ASSOCIATION (PERHIMPUNAN AGRONOMI INDONESIA/PERAGI)
REGIONAL COMMISSIONER OF WEST JAVA
PUBLISHED BY: UNPAD PRESS

JURNAL ***KULTIVASI***

Volume 25 Issue 1 April 2026
ISSN: 1412-4718, eISSN: 2581-138x

ORGANIZED BY
Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran &
Indonesian Agronomy Association (Perhimpunan Agronomi Indonesia/PERAGI) Regional Commissioner of West Java

PUBLISHED BY
Unpad Press

Published in April, August, December each year

ADDRESS
Departemen Budidaya Pertanian
Fakultas Pertanian Universitas Padjadjaran
Gedung Budidaya Pertanian Lt. 3
Jl. Ir. Soekarno Km. 21, Jatinangor, Sumedang 45363
West Java, Indonesia

Website: jurnal.unpad.ac.id/kultivasi
Email: jurnal.kultivasi@unpad.ac.id

PREFACE

The articles in Kultivasi Journal's volume 25, number 1 (2026) originate from a variety of agricultural-related scientific fields. These articles on plant production are from diverse disciplines, including agronomy, soil science, weed science, plant pests and diseases, and plant breeding. Both commercial and early-stage plant output are increased by this information.

The type of our article is an original research paper and an article review based on a comprehensive, up-to-date set of references that has a significant impact on farming knowledge. Experienced editors and reviewers from Indonesia and outside have edited and reviewed the ten articles written by academicians and researchers in this publication. It is expected that these articles will increase the knowledge of academic scientists and agricultural practitioners. Academic and research participation is crucial to the farming industry's future expansion.

This year, Kultivasi Journal remains committed to publishing an English edition. In the near future, it will facilitate and support our journal's international indexing. We appreciate the commitment of readers, writers, and reviewers to our ongoing publication development.

Editorial of Kultivasi Journal

AUTHOR'S INSTRUCTIONS

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

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

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

Special Requirements

Review Articles:

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

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

Research Articles:

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

Title

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

Author's name

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

Abstract

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

Introduction

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

Materials and Method

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

Results and Discussions

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

Conclusion

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

Acknowledgment

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

Reference

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

- Two authors: Tati Nurmala and Yudithia Maxiselly *then written* Nurmala and Maxiselly (2014) or (Nurmala and Maxiselly, 2014).
- Three or more authors: Nurmala et al. (2014) or (Nurmala et al., 2014).
- Book writing format: Author's Last Name. Initials of the year it was published. Book title (each initial letter of a word is written in capital letters, except for conjunctions/prepositions; Edition if the edition is more than one). Name Publisher. Place published.
  Example: Solaimalai A, Anantharaju P, Irulandi S, Theradimani M. 2021. Maize Crop Improvement, Production, and Post Harvest Technology. CRC Press. Oxon.
- Article/Journal writing format: Author's last name. Publication Year initials. Article title (only letters at the beginning of the title are capitalized, except for place names, varieties, and people). Journal name, Volume(Issue): Page number.
  Example: Zakry FAA, Shamsuddin ZH, Rahim KA, Zakaria ZZ, Rahim AA. 2012. Inoculation of Bacillus sphaericus UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the 15N isotope dilution technique. Microbes Environ., 27: 257–262.

CONTENTS

ISSN: 1412-4718, eISSN: 2581-138x

Yuliany NN · Farhan AM · Ziyaroh PL · Dwinianti EF · Nihayah H · Su'udi M

Morphology and bioactive compounds of gurmar (*Gymnema sylvestre*) with potential as antidiabetics

Abstract. The medicinal plant *Gymnema sylvestre* is widely known for its ability to lower blood sugar levels. This study reviewed the physical characteristics and bioactive compounds of *G. sylvestre* sourced from different regions, emphasizing its potential as an antidiabetic agent. Morphological analysis includes leaf structure, trichome presence, and other anatomical features, which can influence the plant's chemical profile. Phytochemical analysis, performed using techniques like GC-MS, identifies key bioactive compounds such as saponins, flavonoids, and gymnemic acids, which contribute to its hypoglycemic effect. The findings indicate that regional variations can affect the concentration and presence of these compounds, suggesting that environmental factors and genetic diversity play significant roles. This comprehensive characterization supports the use of *G. sylvestre* as a natural antidiabetic treatment, which supports its potential as a source of antidiabetic-related bioactive compounds. Further research is recommended to explore the pharmacological mechanisms and optimize the extraction methods for maximum efficacy.

Keywords: Antidiabetic potential · Gymnemic acids · Medicinal plants · Morphological analysis · Phytochemical profiling

Submitted: 2 July 2025, Accepted: 27 January 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.64921

Yuliany NN[1] · Farhan AM[1] · Ziyaroh PL[1] · Dwinianti EF[1] · Nihayah H[1] · Su'udi M[1]*
[1] Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember. Jalan Kalimantan No. 37, Tegalboto, Jember 68121, East Java, Indonesia
*Correspondence: msuudi.fmipa@unej.ac.id

Introduction

Gymnema sylvestre is one of the plants of the Apocynaceae family that is spread across India, Africa, Australia, and China (Khan et al., 2019). *G. sylvestre* lives in several tropical forest areas and tends to like areas that are quite dry, on the surface *G. sylvestre* can be found at a maximum altitude of 600 meters above sea level. This plant has woody stems and is characteristic of its flower shape, so it is a differentiator between *G. sylvestre* and other plants (Vimala et al., 2023). Studies on the morphological characterization of *G. sylvestre* can be developed, this is due to the diverse morphological variation of *G. sylvestre* according to its habitat (Srivastava et al., 2019; Kalariya et al., 2021). One of the different variations of *G. sylvestre* is the morphology of its leaves. *G. sylvestre* leaves are green, oval, and elliptical in shape, and can grow up to 5 cm in length (Tripathi et al., 2024). The morphological diversity of *G. sylvestre* leaves, including their size and color, is believed to be influenced by environmental factors.

The leaves *of G. sylvestre* physiologically have their own uniqueness, because they are often used as traditional medicine in India and China (Khan et al., 2019). Local residents use *G. sylvestre* leaves to make herbal tea. *G. sylvestre* leaves are suspected to have bioactive compounds that have the potential to provide a certain health naturally, one of these properties is as an anti-diabetes mellitus (Gayathri et al., 2018; Thennarasi et al, 2024). *G. sylvestre* leaves contain active compounds such as pentriacontan, phytine, d-queritol, and gymnemic acid, which are known to have anti-sweet properties (Jamadagni et al., 2021). Phytochemically, *G. sylvestre* contains bioactive compounds such as saponins, flavonoids, and terpenoids which are natural remedies and have the potential to be anti-diabetic (Subramanian et al., 2020). The use of the GC-MS chromatography method is one of the recommended methods to identify volatile compounds in *G. sylvestre* (Subramanian et al., 2020).

The research of *G. sylvestre* mostly used samples from India, with the main active compound in the form of gymnemic acids having β-glucuronic groups in C-3 and hydroxyl in C-23 in the oleana triterpene framework (Pham et al., 2018). Gymnemic acids were successfully isolated and purified from *G. sylvestre* revealed that all related molecules exhibit anti-diabetic activity (Kashif et al., 2023). Kashima et al. (2017), suggested that *G. sylvestre* lowers the perception of sweetness and plays a role in slowing down gastrointestinal blood flow as well as gastric emptying, which can affect glycemic metabolism. *G. sylvestre* according to previous studies was identified to have functional proteins, namely, aldose reductase, glucokinase, fructose 1,6-bisphosphatase, cytochrome 450, dipeptidyl peptidases, 11β-hydroxysteroid dehydrogenase, and glutamine fructose-6-phosphate amido transferase. All of these proteins play an active role in sugar reduction, so *G. sylvestre* has the nickname as the "killer of sugar" (Kalariya et al., 2021; Rathore et al., 2016). There are several names for *G. sylvestre* in various languages, including *Periploca of the Woods* in English, *Meshashringi* and *Madhunashini* in Sanskrit, *Kavali* and *Kalikardori* in Marathi, *Dhuleti* and *Mardashingi* in Gujarati, *Adigam* and *Cherukurinja* in Tamil, *Podapatri* in Telugu, and *Sannagerasehambu* in Kannada (Mandal et al., 2024). *Gymnema* comes from the Hindu word "gurmar," which means "sugar killer," because Indians believe that plants of this genus can balance high blood sugar levels in people with diabetes (Ditchou et al., 2024).

Diabetes mellitus (DM) is one of the diseases caused by high sugar levels in the human body, followed by insulin deficiency (Parveen et al., 2019). Diabetes mellitus occurs when the body does not produce enough insulin or cells do not respond well to insulin, causing blood glucose levels to rise (Kumar et al., 2017). Diabetes mellitus is also said to be a chronic hyperglycemia condition characterized by disturbances in lipid, carbohydrate, and protein metabolism (Ditchou et al., 2024). Symptoms of this metabolic disorder include polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive appetite) (Shanmugam et al., 2023). The interaction between genetic factors, environmental conditions, and modern lifestyle habits also influences the development of metabolic disorders, especially in type 2 diabetes (Dar et al., 2024). According to WHO data, diabetes mellitus is one of the deadly diseases with a total of 347 million people, with an estimated 7th cause of death in 2030 (Parveen et al., 2019). Based on previous research, the use of the herb *G. sylvestre* is very potential in the future to limit the use of pharmacological drugs on a regular basis. This study aims to comprehensively examine the morphological character and profile of bioactive compounds of *G. sylvestre* which have the potential as antidiabetic agents by emphasizing the relationship between morphological variations, environmental factors, and phytochemical content. The novelty of this study lies in an integrative approach based on the latest literature review that combines morphology, anatomy, and bioactive compound profile analysis to provide a systematic understanding of the geographic variation of *G. sylvestre* and its implications for potential antidiabetic activity.

Materials and Methods

The research method approach used in this study is systematic scientific review and meta-analysis, starting with topic determination, followed by literature search, journal selection, interpretation of results, and analysis of the impact of conclusions on related fields. The literature search was conducted on scientific databases, namely PubMed, Scopus, and Google Scholar, using a combination of keywords *Gymnema sylvestre*, morphology, anatomy, phytochemical, GC-MS, bioactive compounds, and antidiabetic activity, with a publication period limit between 2015 and 2025. The inclusion criteria include original research articles and review articles that report on morphological or anatomical characters, bioactive compound profiles, phytochemical analysis, as well as the antidiabetic activity of *G. sylvestre* through in vitro, in silico, in vivo, or clinical approaches. In contrast, the articles that are irrelevant, unavailable in full text, or lack adequate scientific data were excluded from the analysis. Articles that meet the criteria were then selected in stages based on titles, abstracts, and complete texts, then descriptive data processing and integration of scientific findings to formulate conclusion that are in accordance with the research objectives. The various effects of *G. sylvestre* species extracts as antidiabetic drugs have been made an overview of newly discovered chemicals isolated from *Gymnema* species over the past five years have also been given (Kahksha et al, 2022). The study selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, which consist of identification, screening, eligibility, and inclusion (Figure 1). The literature selection process was conducted in accordance with the PRISMA principle to ensure transparency and reproducibility.

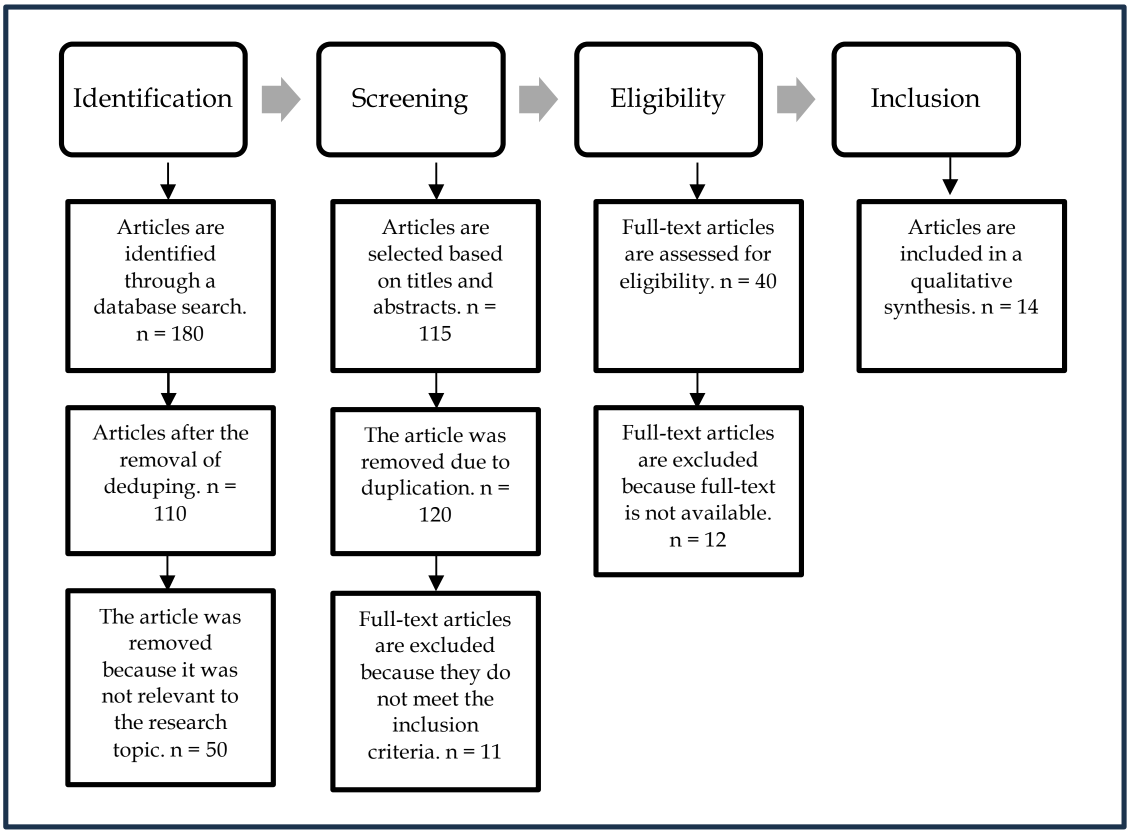


Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) study flow diagram

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

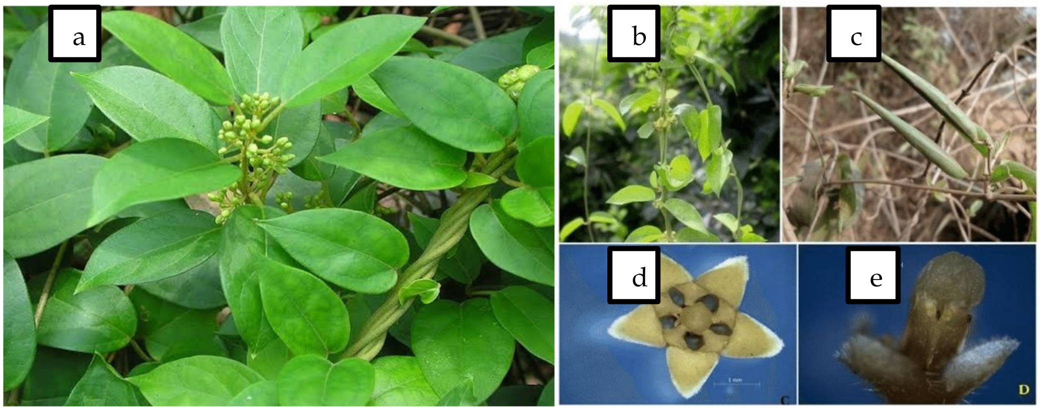


Figure 2. The morphological of *Gymnema sylvestre*. (a) Habitus; (b) Flowering twigs; (c) Fruiting twigs; (d) Light microscope (LM) images of flowers; (e) Gynostegium flowers (Source: Buddhiwant & Mali, 2022; Sinha and Mondal, 2017).

Results and Discussion

Characteristics of *G. sylvestre* *G. sylvestre* has stem organs, flowers, roots, and leaves. The trunk of *G. sylvestre* is long, slender, can creep on surrounding trees, and has a fairly hard texture in old age. The flowers in *G. sylvestre* are yellow, in multiples of 5, and have a slight trichome on the sepals. The leaves are elliptical to tapered, the upper surface is smooth, while the lower surface can be sparsely hairy to tight. Its reproductive structure is characterized by the presence of five fleshy scales on the crown, two upright pollinias, two carpels with one ovarian space, many seeds, and fruits in the form of long fusiform-shaped follicles. The following is a morphological picture of *G. sylvestre* (Figure 2).

G. sylvestre is an annual woody liana that grows in the tropical forests of central and southern India, rooted in the trunk, has elliptical to tapered leaves with varied hairy adaxial and abaxial surfaces, small pale yellow flowers in axillary or lateral umbrella-shaped inflorescences, as well as a distinctive reproductive feature in the form of a campanulate crown with five fleshy scales, two erect polynya, two single-chambered ovarian carpels with many seeds, and fruit in the form of elongated fusiform-shaped follicles (Buddhiwant and Mali, 2022). The morphological and reproductive structure characteristics of *G. sylvestre* belonging to the family Asclepiadaceae are shown through the observation of vegetative organs and flowers (Figure 2). This plant is a perennial woody climber with elliptical to oval face-to-face leaves, small pale-yellow flowers arranged in axillary inflorescences, and fruits in the form of a pair of elongated lanceolate follicles. The structure of the flower shows the characteristic of Asclepiadaceae, namely a bell-shaped crown with a single crown that has five fleshy scales, stamens with modified connectives, and a complex gynostegium (Sinha and Mondal, 2017).

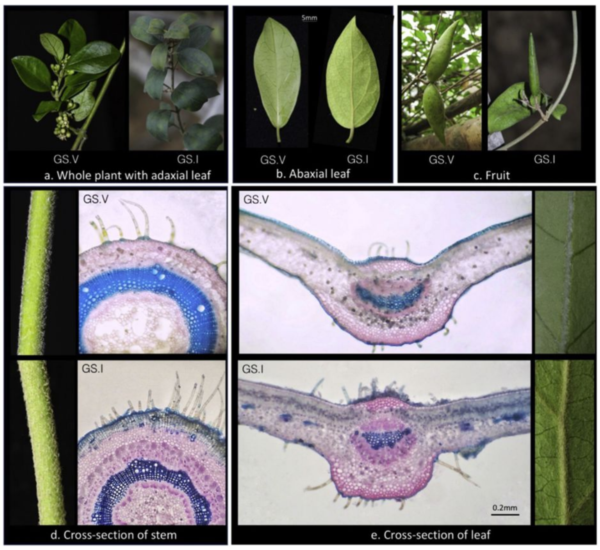


Figure 3. Morphological and anatomical characteristics of 2 varieties of *Gymnema sylvestre*. (Source: Pham et al., 2019).

Comparison of morphological and anatomical characters of *G. sylvestre* from two geographical varieties, namely Vietnam (GS. V) and India (GS. I) (Figure 3). External morphological characters indicate differences in the overall shape of the plant, the leaf surface of the adaxial and abaxial parts, as well as the characteristics of the fruit, which indicate phenotypic variation due to differences in geographical origin. Analysis of stem anatomy through transverse incisions showed variations in the thickness of epidermal tissue, the presence and density of trichomes, and the arrangement of vascular tissue between the two varieties. In addition, the cross-sectional slices of the leaves show differences in the structure of the mesophyll and the vascular system, so the anatomical character can be used as a scientific basis for distinguishing the varieties of *G. Sylvestre* (Pham et al., 2018).

The morphological differences of *G. sylvestre* between varieties of Vietnamese and Indian origin are indicated by variations in leaf color, greenishness, and surface character of vegetative organs. The Vietnamese variety tends to have more intense green leaves and fresher-looking tissues, while the Indian variety shows relatively paler to yellowish leaves, which are related to the different growing environmental conditions, particularly temperature, humidity, and light intensity. These morphological variations reflect the physiological response of plants to changes in temperature and microclimatic conditions that affect chlorophyll content, photosynthesis efficiency, and primary metabolic processes, as reported in various plant species (Bae & Kim, 2023; Jing et al., 2016). Light intensity, light spectrum quality, and photoperiod are known to play an important role in regulating plant morphological development, photosynthetic activity, and carbon metabolism, so these differences in environmental factors are the main basis for *G. sylvestre* morphological variation between Vietnamese and Indian varieties (Tang et al., 2022).

Based on this morphology, it appears that *G. sylvestre* in areas of brighter sunlight intensity has more trichomes (Karabourniotis et al., 2020). The presence of trichomes is very important for *G. sylvestre*, they can functionally help the adaptation process of the plant to the environment (Guan et al., 2022; X. Wang et al., 2021). Theoretically, plants living in warmer regions have a higher number of trichomes, which is in line with the function of trichomes, namely to minimize water evaporation in plants (Wang et al., 2021), so that *G. sylvestre* in areas with fairly high sunlight intensity does not lose too much water. The presence of water in a plant is very important to be used in metabolic processes such as photosynthesis, this also applies to *G. sylvestre* (Wu et al., 2022). The difference in the number of trichomes in *G. sylvestre* is unique in itself to be studied in more depth. Trichomes are functionally intended to prevent water evaporation in plants, but trichomes also have a correlation with the activity of bioactive compounds in plants (Guan et al., 2022).

.

Table 1. The list of major bioactive compounds identified in *G. sylvestre* from India was determined using GC-MS analysis

Peak	Compound	RT (min)	%total	Activity	Reference
1.	n-Hexadecanoic acid	28.814	5.186	Anticancer, antidiabetic	(Aslam et al., 2024; Ravi & Krishnan, 2016)
2.	Phytol	30.763	10.294	Antioxidant, anticancer, antimicrobial, anti-inflammatory	(Kumosani et al., 2024)
3.	Squalene	39.274	10.282	Antioxidant and Anti-inflammatory	(Micera et al., 2020)
4.	Tetratriacontane	44.315	9.570	Antioxidants and antimicrobials	(Hepokur et al., 2020)
5.	Stigmasterol	48.979	2.484	Antidiabetic	(Bakrim et al., 2022; Wang et al. 2017)

(Source : Subramanian et al., 2020).

Bioactive Compound Content of *G. sylvestre* and Its Potential. Based on the literature studies that have been conducted, there are several factors that affect the existence of a bioactive compound contained in a plant extract, one of which is environmental factors. In the study that has been conducted by Subramanian et al., (2020) is the analysis of GC-MS on two samples of *G. sylvestre* from India (representative of the abundant number of trichomes and located in the contours of higher light intensity) (Table 1)

The results of the GC-MS analysis identified that the *G. sylvestre* extract sample from India had bioactive compounds with different percentages of total ingredients. The dominant bioactive compounds found in *G. sylvestre* are squalene (10%), phytol (10%), tetratriacontane (9%), n-hexadecanoic acid (5%), and stigmasterol (2%). All of these bioactive compounds have their own activities. The detected bioactive compounds have a dominant total percentage, have potential biological activity as antioxidants, antimicrobials, anti-inflation, anticancer, and antidiabetics. Squalene and phytol are bioactive compounds that have the highest percentage of total (10%), according to the literature squalene has the ability of biological activity as an antioxidant and anti-inflammatory. Squalene (SQ) is a metabolite compound that belongs to the triterpene group. The compound has the chemical formula C30H50 and is high in antioxidants. The activity of squalene as an antioxidant, if applied in the medical field has potential as a prevention of hypertension and hypercholesterolemia. Squalene in the GC-MS *G. sylvestre* test can be found at the 31st peak (Micera et al., 2020).

Phytol is one of the bioactive compounds detected in *G. sylvestre* at the 23rd peak. Phytol compounds are one of the potential compounds that have anticancer properties (Kumosani et al., 2024). The mechanism of phytol compounds as anticancer is to inhibit ROS production through NADH oxidase, this is because if ROS production is too excessive, it can support the growth of foreign cells or cancer. Phytol also inhibits the proliferation and regulation of genes, that is, affects protein kinase pathways such as ERK1/2 and JNK. Disruption of these gene pathways can prevent the proliferation and growth of cancer cells. Theoretically, phytols are also able to perform apoptosis in foreign cells, namely by increasing the release of cytochrome C (CYP-C) in the mitochondrial pathway, so that it can activate the enzyme capase-3 which functions as an executor in the apoptosis pathway (Alencar et al., 2018).

The molecular mechanism of phytol compounds as anticancer agents through cellular signaling pathways. Phytol interacts directly with transmembrane receptors such as RTK (receptor tyrosine kinase), GPCR (G protein-coupled receptor), and CKR (chemokine receptor), activating the PI3K and PKC signaling pathways, as well as influencing the activation of transcription factor NF-κB to regulate gene expression related to cancer cell survival and cellular migration via MMPs (matrix metalloproteinases). The production of reactive oxygen species (ROS) is increased through the activation of NADPH oxidase, causing oxidative stress and mitochondrial damage, which triggers the release of cytochrome c (CYP-c). The activation of this mitochondrial pathway induces caspase 9 and caspase 3, which directly initiate apoptosis. This apoptotic pathway is reinforced by the inhibition of anti-apoptotic proteins such as Bcl-xL and increased expression of pro-apoptotic proteins such as Bax. Gene regulation is affected through the ERK1/2 and JNK signaling pathways, which can modulate gene transcription, stop cell cycles, or induce apoptosis depending on the context. Activation of the p53, p38, p21, and p16 proteins also contributes to the control of cancer cell proliferation. An additional mechanism involves the activation of the RIP and MLKL proteins, which trigger non-programmed cell death through necrosis (Alencar et al., 2018).

Tetratriacontane is one of the dominant bioactive compounds found in *G. sylvestre* with a total percentage of 9%. Tetratriacontane biologically has potential, one of which is activity as an antimicrobial (Hepokur et al., 2020). The compound may support *G. sylvestre* as an antimicrobial. According to the results of research by Gayathri et al. (2018) *G. sylvestre* proved to have potential as an antimicrobial by being able to inhibit the growth rate of specific microbes of gram-positive bacteria, namely *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*, in addition to gram-positive bacteria, *G. sylvestre* can inhibit gram-negative bacteria, namely *Escherichia coli bacteria*, *Shigella flexneri* and *Proteus vulgaris*. The presence of a clear zone in the bacterial culture medium indicates inhibition.

The compounds n-Hexadecanoic acid (peak-20) and Stigmasterol (peak-35), are both potential compounds in *G. sylvestre* that have the same biological activity, namely as an antidiabetic. Hexadecanoic acid is one of the compounds of hexane, which is often found in plant samples including *G. sylvestre*. Research on specific n-Hexadecanoic acid applied to antidiabetes has not been widely done and needs to be further developed (Aslam et al., 2024). Stigmasterol compounds have begun to be developed in the application of antidiabetic mellitus. Stigmasterol has potential as an antidiabetic with evidence it can help improve the regulation of glucose levels in the blood and improve insulin sensitivity. Research shows that the administration of stigmasterol increases the translocation of GLUT4 (glucose transporter type 4) and the regeneration of cells β the pancreas, which plays a role in insulin secretion. This effect helps lower fasting glucose levels and improves oral glucose tolerance, which is important for the management of type 2 diabetes (Wang et al., 2017). The potential of *G. sylvestre* as an antidiabetes is unique, because in several Asian countries it has taken a traditional approach to use *G. sylvestre* as an antidiabetic drug, one of these countries is India (Rathore et al., 2016).

Overall, the bioactive compound content in *G. sylvestre* has different biological activity capabilities, but further research needs to be conducted in order to confirm the primary data regarding the profile of each bioactive compound.

Potential of *G. sylvestre* as an Antidiabetes. *G. sylvestre* in India is believed to be an herbal alternative medicine as an antidiabetes mellitus. Scientifically, according to Laha & Paul, (2019), *G. sylvestre* contains secondary metabolites that are characteristic of the plant, namely gymnemic acid. The GC-MS test has been carried out by (Subramanian et al., 2020) but has not been able to detect the presence of gymnemic acid. Gymnemic acid is specifically a member of the triterpenoid group. Gymnemic acid has several types, namely, gymnemic acid (I-XVIII) homologue, gymnemic acid A1 and its derivatives, and there may still be several others (Laha & Paul, 2019). Gymnemic acid is suspected to be able to prevent the occurrence of diabetes mellitus because it can inhibit sugar absorption, so in countries such as India and China the process of developing the research (Gaytán Martínez et al., 2021). The research stage starts from in silico gymnemic acid testing. The results of the study of Shenoy et al., (2021) indicate that Gymnemic acid I has an interaction with the enzyme Sucrase α-glucosidase (one of the sugar-binding enzymes). The in silico interaction can be continued in the in vitro stage, because gymnemic acid I can produce binding affinity. (Table 2).

Table 2. In silico molecular docking analysis of gymnemic acid

Enzyme	Compounds in *Gymnema*	Residue	Binding Affinity
Sucrase α-glucosidase	Gymnemic Acid I	PHE140,PHE244 ARG278,ASP280	-95.8172
	Gymnemic acid IV	VAL325,ASP392, HIS443, ARG515	-101.119

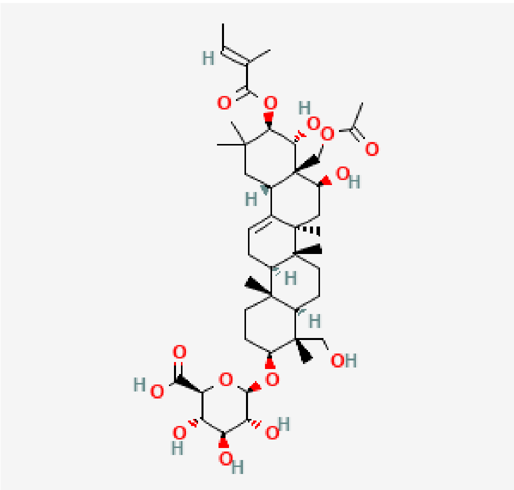
(Source : Shenoy et al., 2021).

The chemical structure of gymnemic acid I and gymnemic acid IV, the two main triterpenoid glycoside compounds isolated from *G. sylvestre* (Figure 4). Both compounds have a hydrophobic oleanane-type triterpene base structure and are conjugated to sugar groups through glycosidic bonds, which contribute to increased solubility and biological activity. Gymnemic acid I is composed of gymnemagenin aglicon containing several hydroxyl and carboxyl groups, which play an important role in forming molecular interactions with target enzymes of carbohydrate metabolism. Gymnemic acid IV has differences in the amount, position, and type of sugar substituents as well as functional groups, resulting in variations in molecular conformation and chemical properties. These structural differences affect the binding affinity of the compounds to the enzymes α-glucosidase and sucrase, which is indicated by a higher binding affinity value in gymnemic acid IV than in gymnemic acid I.

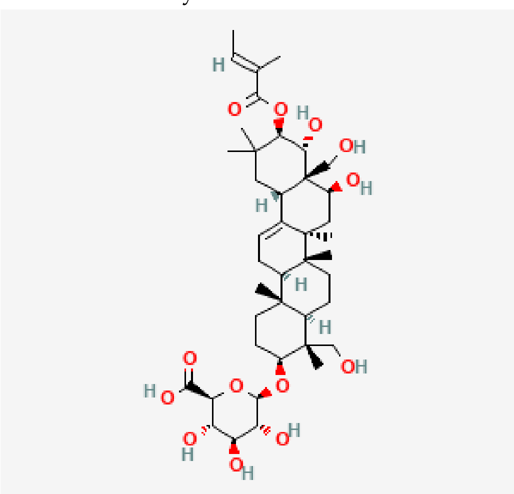
Kumar et al. (2010) reported the results of a study on patients with type 2 diabetes mellitus treated with *G. sylvestre* 500 mg per day for three months. Treatment results showed a decrease in fasting blood glucose levels, postprandial blood glucose, and glycated hemoglobin, as well as improvements in symptoms of fatigue, polyphagia, and lipid profile. Devangan et al. (2021) conducted a systematic review and meta-analytical study that included 10 studies with a total of 419 patients with type 2 diabetes. This study showed that the treatment of *G. sylvestre*

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

was associated with a decrease in fasting blood sugar ($P < 0.0001$), postprandial blood sugar ($P < 0.0001$), and glycosylated hemoglobin ($P < 0.0001$). Treatment also significantly lowers blood triglycerides ($P < 0.0001$), and cholesterol ($P < 0.0001$). The available evidence suggests that *G. sylvestre* is a hypoglycemic agent and lipid lowering agent. However, the available evidence research also shows the usefulness of several other supplements including fenugreek, cinnamon, and lipoic acid in the management of diabetes. Therefore, the choice of diabetes supplements depends to some extent on availability and cost. It appears that *G. sylvestre* is the most available and cost-effective diabetes supplement in India, however, it is not present in any other country in the world including Iraq (Alam et al., 2022).



Gymnemic acid I



Gymnemic acid IV

Figure 4. Structure of gymnemic acid compounds I and IV (PubChem, 2025)

Conclusion

This overview highlighted that *G. sylvestre* exhibits morphological variation across different countries, as reported in the reviewed literature, with differences in vegetative traits descriptively linked to environmental factors, particularly light intensity. However, since this study relies on secondary data regardless controlled experimental observations, the relationship needs to be interpreted carefully and requires further confirmation through supporting experimental studies. *G. sylvestre* has great potential as a natural medicinal agent, this is due to the content of bioactive compounds in *G. sylvestre* which are abundant and have biological activities such as antioxidants, anticancer, antimicrobials, and natural antidiabetics. One of the potential ingredients of *G. sylvestre* is gymnemic acid which provides hypoglycemic effects as an antidiabetic. This report was constructed as a literature-based synthesis study that integrates the latest findings regarding the morphological character, bioactive compound content, and antidiabetic potential of *G. sylvestre*, thus providing a comprehensive framework of understanding as a scientific basis for further research. This study is expected to be a conceptual reference in the development of experimental research and pharmacological applications of *G. sylvestre* in the future.

References

Alam O, Naaz S, Sharma V, Manaithiya A, Khan J, Alam A. 2022. Recent developments made in the assessment of the antidiabetic potential of Gymnema species-From 2016 to 2020. Journal of ethnopharmacology, 286, 114908.

Alencar MVOB, Islam MT, Ali ES, Santos JVO, Paz MFCJ, Sousa JMMC, Dantas SMMM, Mishra SK, Cavalcante AACM. 2018. Association of Phytol with Toxic and Cytotoxic Activities in an Antitumoral Perspective: A Meta-Analysis and Systemic Review. Anti-Cancer Agents in Medicinal Chemistry, 18(13): 1828–1837.

Aslam T, Arif A, Arshad S, Muccee F, Ahmad K, Iqbal MO, Khalil U, Razak S, Afsar T, Almajwal A, Shafique H, Zain M. 2024. Discovering the anti-diabetic potential of pomegranate peel metabolites by examining molecular interplay with the thioredoxin-interacting protein. Frontiers in Medicine, 10(September).

Bae H, Kim J. 2023. Exploring the Mechanisms of Humidity Responsiveness in Plants and Their Potential Applications. Applied Sciences (Switzerland), 13(23).

Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, Sheikh RA, Goh KW, Ming LC, Bouyahya A. 2022. Health Benefits and Pharmacological Properties of Stigmasterol. Antioxidants, 11(10): 1–32.

Buddhiwant RS, Mali SR. 2022. Review on Gymnema Sylvestre as an Major Antidiabetic Agent. International Journal of Research Publication and Reviews, 3 (6): 2815 – 2839.

Dar MI, Sultan A, Abass S, Dev K, Parveen R, Ahmad S, Qureshi MI. 2024. Exploring the anti-diabetic mechanism of selective phytochemicals identified from Gymnema sylvestre using TLC-UPLC-MS, complemented by in silico studies. Phytomedicine plus, 4(3): 100606.

Ditchou YON, Leutcha PB, Miaffo D, Mamoudou H, Ali MS, Ngnoung GAA, Soh D, Agrawal M, Darbawa R, Tchouboun EZN, Lannang AM, Noundou XS. 2024. In vitro and in silico assessment of antidiabetic and antioxidant potencies of secondary metabolites from Gymnema sylvestre. Biomedicine & Pharmacotherapy, 177, 117043.

Gayathri S, Sivaraj C, Sangeetha S, Arumugam P, Manimaram A. 2018. Evaluation of antioxidant, antibacterial, alpha amylase enzyme inhibition activities and GC-MS analysis of leaves extract of Gymnema sylvestre L. Journal of Pharmacognosy and Phytochemistry, 7(6): 2326–2333.

Gaytán Martínez LA, Sánchez-Ruiz LA, Zuñiga LY, González-Ortiz M, Martínez-Abundis E. 2021. Effect of Gymnema sylvestre Administration on Glycemic Control, Insulin Secretion, and Insulin Sensitivity in Patients with Impaired Glucose Tolerance. Journal of Medicinal Food, 24(1): 28–32.

Guan Y, Chen S, Chen F, Chen F, Jiang Y. 2022. Exploring the Relationship between Trichome and Terpene Chemistry in Chrysanthemum. Plants, 11(11): 1–14.

Hepokur C, Misir S, Tunc T, Tutar U, Çiçek M. 2020. In Vitro antimicrobial, antioxidant, cytotoxic activities, and wound healing potential of Thymbra capitata ethanolic extract. Turkish Journal of Biochemistry, 45(6): 843–849.

Jamadagni PS, Pawar SD, Jamadagni SB, Gautam M, Gaidhani SN, Prasad GP, Gurav AM. (2021). Recent Updates in Research on Gymnema sylvestre. Pharmacognosy Reviews, 15(30).

Jing P, Wang D, Zhu C, Chen J. 2016. Plant physiological, morphological and yield-related responses to night temperature changes across different species and plant functional types. Frontiers in plant science, 7, 1774.

Kalariya KA, Meena RP, Poojara L, Shahi D, Patel S. 2021. Characterization of squalene synthase gene from Gymnema sylvestre R. Br. Beni-Suef University Journal of Basic and Applied Sciences, 10(1): 0–10.

Kahksha, Alam O, Naaz S, Sharma V, Manaithiya A, Khan J, Alam A. 2022. Recent developments made in the assessment of the antidiabetic potential of Gymnema species - From 2016 to 2020. Journal of ethnopharmacology, 286, 114908.

Karabourniotis G, Liakopoulos G, Nikolopoulos D, Bresta P. 2020. Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. Journal of Forestry Research, 31(1): 1–12.

Kashif M, Nasir A, Gulzaman, Rafique MK, Abbas M, Rehman AU, Riaz M, Mtewa AG. 2023. Unlocking the anti-diabetic potential of Gymnema sylvestre, Trigonella foenum-graecum, and their combination thereof: An in-vivo evaluation. Food Science & Nutrition, 11(12): 7664-7672.

Kashima H, Eguchi K, Miyamoto K, Fujimoto M, Endo MY, Aso-Someya N, Kobayashi T, Hayashi N, Fukuba Y. 2017. Suppression of oral sweet taste sensation with Gymnema sylvestre affects postprandial gastrointestinal blood flow and gastric emptying in humans. Chem. Senses 42, 295e302.

Khan F, Sarker MMR, Ming LC, Mohamed IN, Zhao C, Sheikh BY, Tsong HF, Rashid MA. 2019. Comprehensive review on phytochemicals, pharmacological and clinical potentials of Gymnema sylvestre. Frontiers in Pharmacology, 10(OCT): 1–19.

Kumar P, Rani S, Arunjyothi B, Chakrapani P, Rojarani A. 2017. Evaluation of antidiabetic activity of Gymnema sylvestre and Andrographis paniculata in streptozotocin induced diabetic rats. Int J Pharmacogn Phytochem Res, 9(1): 22-5.

Kumosani TA, Alnefayee A, Barbour E, Qari M, Ahmed T, Moselh SS. 2024. Identification of Bioactive Ingredients of Traditional Medicinal Plants Psiadia arabica Jaub. Tamarix articulata, Terminalia arjuna and Rhazya stricta by GC-MS in Saudi Arabia. Pharmacognosy Research, 16(3): 570–573.

Laha S, Paul S. 2019. Gymnema sylvestre (Gurmar): A potent herb with anti-diabetic and antioxidant potential. Pharmacognosy Journal, 11(2): 201–206.

Mandal SK, Rahmat S, Sakib K, Mehjabin B, Rahman T, Rasna IJ. 2024. An assessment of anti-diabetic effect of Gymnema sylvestre in alloxan-induced rat model. International Research Journal of Gastroenterology and Hepatology, 7(1): 29-36.

Micera M, Botto A, Geddo F, Antoniotti S, Bertea CM, Levi R, Gallo MP, Querio G. 2020. Squalene: More than a step toward sterols. Antioxidants, 9(8): 1–14.

Parveen S, Ansari MHR, Parveen R, Khan W, Ahmad S, Husain SA. 2019. Chromatography Based Metabolomics and in Silico Screening of Gymnema sylvestre Leaf Extract for Its Antidiabetic Potential. Evidence-Based Complementary and Alternative Medicine, 1, 7523159.

Pham HTT, Hoang MC, Ha TKQ, Dang LH, Tran VO, Nguyen TBT, Lee CH, Oh WK. 2018. Discrimination of different geographic varieties of Gymnema sylvestre, an anti-sweet plant used for the treatment of type 2 diabetes. Phytochemistry, 150: 12-22.

PubChem. Gymnemic acid I and Gymnemic acid IV. https://pubchem.ncbi.nlm.nih.gov. Accessed Jan. 15, 2025.

Rathore PK, Arathy V, Attimarad VS, Kumar P, Roy S. 2016. In-silico analysis of Gymnemagenin from Gymnema sylvestre (Retz.) R.Br. with targets related to diabetes. Journal of Theoretical Biology, 391: 95–101.

Ravi L, Krishnan K. 2016. Cytotoxic Potential of N-hexadecanoic Acid Extracted from Kigelia pinnata Leaves. Asian Journal of Cell Biology, 12(1): 20–27.

Shanmugam SK, Singh M, Kumar D, Mishra R, Barman M. 2023. Exploring Antidiabetic Potential of Gymnema sylvestre. Journal of Applied Pharmaceutical Sciences and Research, 6(2): 30-35.

Shenoy RS, Kumar DJ, HG N, Manonmani HK. 2021. In silico evaluation of naturally isolated Triterpene Glycosides (TG) from Gymnema sylvestre towards diabetic treatment. Heliyon, 7(12): e08407.

Sinha S, Mondal AK. 2017. A phylogenetic study of floral morphology of some members of Asclepiadaceae R. Br. Annals of Plant Sciences, 6(2), 1546-1559.

Srivastava A, Bhatt R, Khobra R. 2019. Gymnema sylvestre. Medicinal properties and biological action. Medicinal Plants in India: Importance and Cultivation, June, 231–248.

Subramanian S, Dowlath MJH, Karuppannan SK, Saravanan M, Arunachalam KD. 2020. Effect of solvent on the phytochemical extraction and GC-MS analysis of Gymnema sylvestre. Pharmacognosy Journal, 12(4): 749–761.

Tang W, Guo H, Baskin CC, Xiong W, Yang C, Li Z, Song H, Wang T, Yin J, Wu X, Miao F, Zhong S, Tao Q, Zhao Y, Sun J. 2022. Effect of Light Intensity on Morphology, Photosynthesis and Carbon Metabolism of Alfalfa (Medicago sativa) Seedlings. Plants, 11(13).

Thennarasi M, Aishwarya S, Vignesh K, Sundararajan S. 2024. A review on Anti-diabetic siddha herbs in management of Diabetes mellitus. European Journal of Biomedical, 11(9): 308-314.

Tripathi A, Pandey A, Khan A. 2024. Gymnema sylvestre: A medicinal plant with versatile therapeutic. 8(3): 1–7.

Vimala C, Ancy S, Mala G, Reginald Appavoo M, Irene Wilsy J. 2023. Gymnema sylvestre (Retz.) R.Br. Ex Sm.: A Botanical, Ethnopharmacological, Phytochemical, And Pharmacological Overview. Journal of Pharmaceutical Negative Results, 14(2): 1152–1157.

Wang J, Huang M, Yang J, Ma X, Zheng S, Deng S, Huang Y, Yang X, Zhao P. 2017. Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food and Nutrition Research, 61(1).

Wang X, Shen C, Meng P, Tan G, Lv L. 2021. Analysis and review of trichomes in plants. BMC Plant Biology, 21(1): 1–11.

Wu J, Wang J, Hui W, Zhao F, Wang P, Su C, Gong W. 2022. Physiology of Plant Responses to Water Stress and Related Genes: A Review. Forests, 13(2)

ISSN: 1412-4718, eISSN: 2581-138x

Sevirasari N · Hidayati W · Az-Zahroh Z · Febriansyah B · Fiana MA · Rochmaniah NN · Faozi K · Bayyinah LN · Maryanto J · Fauzi A

Growth response and yield performance of upland rice intercropped with legumes

Abstract. Intercropping patterns in upland rice must be carefully managed to prevent excessive competition among crops. This research aimed to determine the ideal proportion of upland rice–legume intercropped and legume types for rice growth and yield. The research was conducted in Mersi, East Purwokerto, Banyumas, Central Java, from April–August 2025. The study was arranged in a split-plot design consisting of a main factor in form of the proportion of upland rice to legumes (1:1, 2:1, and 3:1), and a sub-factor, i.e., legume types (peanuts, mung beans, and soybeans). The data were analyzed using Analysis of Variance and Tukey's HSD test at α = 5%. The results showed that the height of upland rice plants in intercropping was significantly higher than in sole cropping at 35 and 56 days after planting (DAP). The SPAD leaf greenness index of upland rice leaves at 70 DAP in sole cropping was significantly higher than in intercropping. Intercropping produced the insignificant number of stems, leaves, panicles, dry weight, number, and weight of grain per plant as upland rice in sole cropping. The number and weight of empty grains per plant, as well as upland rice productivity in sole cropping, were significantly higher than in intercropping. Both factors did not significantly affect the growth and yield of upland rice under intercropping condition. Intercropping upland rice and peanuts at a 2:1 planting proportion resulted in a land equivalent ratio greater than 1, indicating that the system was productive and efficient despite a high competitive ratio.

Keywords: Competitive ratio · Land equivalent ratio · Legume types · Peanuts · Upland rice productivity

Submitted: 21 October 2025, Accepted: 27 April 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.67508

Sevirasari N* · Hidayati W[1] · Az-Zahroh Z[2] · Febriansyah B[1] · Fiana MA[1] · Rochmaniah NN[1] · Faozi K[1] · Bayyinah LN[1] · Maryanto J[3] · Fauzi A[4]
[1] Agronomy and Horticulture Laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University. Jl. Dr. Soeparno 61 Karangwangkal, Purwokerto 53123, Central Java, Indonesia
[2] Plant Breeding and Biotechnology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University. Jl. Dr. Soeparno 61 Karangwangkal, Purwokerto 53123, Central Java, Indonesia
[3] Soil science laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University. Jl. Dr. Soeparno 61 Karangwangkal, Purwokerto 53123, Central Java, Indonesia
[4] Agroecology laboratory, Department of Agrotechnology, Faculty of Agriculture, Jenderal Soedirman University. Jl. Dr. Soeparno 61 Karangwangkal, Purwokerto 53123, Central Java, Indonesia
*Correspondence: nindy.sevirasari@unsoed.ac.id

Sevirasari N, Hidayati W, Az-Zahroh Z, Febriansyah B , Fiana MA, Rochmaniah NN, Faozi K, Bayyinah LN, Maryanto J, Fauzi A. 2026. Growth response and yield performance of upland rice intercropped with legumes. Jurnal Kultivasi, 25 (1): 11-27.

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Upland has great potential to be developed and its productivity increased through agricultural activities, supported by the increasing demand for food in Indonesia, which continues to increase (Benu et al., 2023). The use of upland in agriculture plays a role in helping to increase national food production, one of which is through the planting of upland rice. Upland rice cultivation through the Integrated Crop Management approach is an approach to rice cultivation that is efficient in the use of inputs and pays attention to the wise use of natural resources with the aim of increasing productivity and farmer income, as well as maintaining the sustainability of the production environment through the integrated management of land, water, plants, plant pests, and climate (Rambe et al., 2024).

Upland areas where upland rice is cultivated generally have limited water and low soil fertility (Jarrar et al., 2023). It is known that over the past 5 years (2018–2022), the average rainfall in Banyumas Regency has decreased, especially in July and August during the dry season, from an average of 80 mm to 54 mm (Peraturan Daerah Kabupaten Banyumas Nomor 12 Tahun 2024, 2024). This will affect water availability in uplands that rely solely on rainwater. Uplands generally have low organic matter content with high soil acidity (acidic pH) (Winazira et al., 2021). One approach to increasing upland productivity is the implementation of soil conservation practices (Heryani & Rejekiningrum, 2020). Biological soil conservation can be carried out through cover crop planting methods and intercropping patterns (Bukovsky-Reyes et al., 2019; Pokharel et al., 2023). Intercropping is a method of planting two or more different types of crops on the same land for mutual benefit (Beets, 2019). Crop intensification occurs in both temporal and spatial dimensions when two or more crops are grown concurrently on the same field (Andrews & Kassam, 1976). In dry farming conditions, intercropping systems are typically low-input and risk-reducing for crop diversification and meeting subsistence goals (Nandhini & Somasundaram, 2020).

The potential for upland rice in Banyumas Regency is quite promising for development in 10 subdistricts, namely Wangon, Rawalo, Kebasen, Somagede, Kalibagor, Banyumas, Ajibarang, Gumelar, Pekuncen, and Kedungbanteng. Spatially, Banyumas has zones that are relatively suitable for the development of upland rice, particularly in sub-districts dominated by dryland or transitional rice-fallow agroecosystems (Saraswati et al., 2006). Data from the Banyumas Central Statistics Agency (BPS) for 2023 shows that the area of upland rice fields was 202 hectares, with a production of 893.24 tons and an average yield of 4.42 tons per hectare. Harvesting is concentrated in Lumbir (68 ha; 302.60 tons), Wangon (14 ha; 60.90 tons), Kebasen (29 ha; 130.00 tons), Kalibagor (84 ha; 369.60 tons), and Ajibarang (7 ha; 30.14 tons) (BPS, 2023).

From an agronomic perspective, Banyumas Regency is well-suited for developing rice–legume intercropping, as the combination of suitable land, rainfall patterns, and a large rice production base makes this system more viable than continuing to rely on rice sole cropping. Banyumas Regency also has a strong legume base, so intercropping can utilize crops that farmers are already familiar with. A study on upland rice farming in Ajibarang indicates that approximately 70% of the area consists of upland fields, and that upland rice cultivation on these fields is economically viable, with a benefit-cost ratio of 1.36 (Saraswati et al., 2006). Legume cultivation in Banyumas Regency is widespread and continues to expand, as evidenced by official data on harvested area and production for 2023 for soybeans, mung beans, and peanuts, as well as by the results of a study on priority commodities that identifies several legumes as potential or priority crops in a number of subdistricts in Banyumas (BPS Kabupaten Banyumas, 2026; Destiningsih, 2016).

Among the existing intercropping systems, cereal-legume intercropping is an important and profitable combination because this system has a high potential to reduce inputs such as fertilizers, improve environmental quality, and reduce nutrient emissions, especially in developing countries, where the use of fertilizer inputs and high nutrient emissions in the atmosphere (Raza et al., 2020). Intercropping with legumes increases the accumulation of nutrients, especially nitrogen, plant growth, and crop yields Raza *et al.* (2023). Intercropping with legumes increases the growth and yield components of red rice during irrigation water shortage conditions (Arifuddin et al., 2019).

Setting the proportion of plants is important to know whether the model in the cropping system used is profitable compared to sole cropping, and minimizes competition for space and light distribution (Feng et al., 2019) between upland rice and legume plants. Intercropping rice with legumes can minimize the impact of pest and disease attacks compared to the use of chemical pesticides Iwuagwu *et al.* (2019). According to Assefa & Bitew (2023), the ideal intercropping proportion of lowland rice–uci beans (*Lathyrus sativus* L.) is 3 : 1, with a maximum rice production efficiency of 5.1 tons/ha. The proportion of 75% rice and 25% soybeans produces a higher land equivalent coefficient and area time equivalent ratio compared to the proportion of 50% : 50% (Ozioma et al., 2024).

Legumes are planted after upland rice so that the shade does not hinder the early growth of rice, and by the time the legumes grow, the rice is already tall enough (Galo, 2025). The rice root system is already strong enough, and competition for light and nutrients can be controlled (Papong & Cagasan, 2020), and the efficiency of space and light utilization becomes more optimal (Assefa & Bitew, 2023). Legumes were intercropped after the upland rice plants were 3 weeks after planting (WAP) (Arifuddin et al., 2019). Upland rice intercropped with *C. breviflora,* where beans are planted 25 days after rice is planted, does not hinder the growth and yield of rice (Meirelles et al., 2024).

Intercropping performance is strongly influenced by relative planting density, temporal niche differentiation, and relative height differences among plants; that is, the biological effects of planting proportions may depend on the companion plants' species identity (Ruillé & Beillouin, 2026). This study focuses on the ability to detect differences among legume species and how these species modify the effects of proportions. Intercropping optimization is strongly influenced by species combinations, crop ratios, and sowing densities; therefore, the selection of levels for the legume species factor must be based on the agronomic and ecological differences among species (Zustovi et al., 2024). Based on this, the selection of peanuts, mung beans, and soybeans as sub-plot levels is justified because all three represent three different types of legumes in terms of root architecture, drought stress tolerance, growth duration, water requirements during the generative phase, and potential contribution to system complementarity (Afonso et al., 2025). Peanuts were selected because they are more drought-tolerant, have a strong root system, and can explore the soil effectively, thereby potentially enhancing complementarity with upland rice (Gelaye et al., 2025). Mung beans were selected because they have a short growth period and possess drought escape mechanisms, although they remain sensitive during the flowering and pod-filling stages (Singh et al., 2021). Soybeans were selected because they have higher water requirements and are more sensitive to drought stress, particularly during the reproductive stage, thus representing legumes with greater resource demands (Wang et al., 2022). This study aims to determine the optimal intercropping proportion between upland rice and legumes, as well as the most suitable legume species (peanuts, mung beans, and soybeans), to improve rice growth and yield, thereby generating appropriate technology that can be adopted by farmers.

Materials and Methods

Study area. This study was conducted from April to August 2025 in the experimental field, Mersi, East Puswokerto, Banyumas Regency, Central Java Province, Indonesia, located at 7°25′51″S, 109°16′1″E, and an altitude of ±80.9 m above sea level (asl). Morphological and post-harvest analysis were conducted in the Agronomy and Horticulture Laboratory, Faculty of Agriculture, Jenderal Soedirman University.

Experimental design. This research aimed to determine the ideal proportion of upland rice intercropped with legume and legume types for rice growth and yield. The materials used were Situ Bagendit upland rice variety, mung beans (VIMA 5), peanuts (Lurik), and soybeans (Grobogan), Arbuscular Mycorrhizal Fungi, Legume Inoculant, Urea fertilizer (N: 46%, Kaltim), SP-36 (P_2O_5: 36%, S: 5%, PETRO) fertilizer, KCl (K_2O: 60%, PETRO), herbicides post-emergent, insecticide, fungicide, and dolomite.

The research was arranged by a split-plot experimental design with two treatment factors and three blocks as replicates. The main factor was the proportion of upland rice rows to legume rows, consisting of three levels: 1:1, 2:1, and 3:1 rows. The sub-plots were planting patterns consisting of 3 levels: rice + peanuts, rice + mung beans, rice + soybeans. Sole cropping patterns of rice, peanuts, mung beans, and soybeans served as controls. Each experimental plot unit covers an area of 4 m² (2 m x 2 m), with a planting distance of 25 cm x 25 cm for rice (Paiman et al., 2023) and a row spacing of 40 cm x 15 cm for peanuts (Hawalid, 2019) and mung beans (Amanullah et al., 2022), and 40 cm x 20 cm for soybeans (Harsono et al., 2021). In intercropping, the distance between legumes is adjusted according to the proportion being tested, while the distance within rows is the same as the distance within rows in sole cropping, to reduce competition between the two plant species.

There were 9 treatment combinations and 4 control treatments. The total land area was 156 m² (4 m² x 9 units x 3 blocks + 4 control units x 3 blocks). One experimental plot unit contained 64 rice plants, and the experimental units with a ratio of upland rice to legumes of 1:1 contained 91 peanut and mung bean plants, and 70 soybean plants. The ratio of upland rice to legumes was 2:1, with 52 peanuts and mung beans, and 40 soybeans. The ratio of upland rice to legumes was 3:1, with 39 peanuts and mung beans, and 30 soybeans. The peanut and mung bean sole cropping population was 65 plants, and the soybean sole cropping population was 50 plants. A schematic of the cropping model could be seen in Figure 1.

Figure 1. Model of the proportion of intercropping of legume rows : rice (a) 1: 1, (b) 1: 2, (c) 1: 3. Rice planting distance 25 cm x 25 cm.

Research procedure. The research site was a taro field (*Colocasia esculenta*). The soil was thoroughly plowed to loosen and rid the soil of weeds. Experimental plot units were made with a size of 2 m (length) x 2 m (width) for 27 experimental plot units for intercropped and 12 experimental plot units for sole cropping. Rice seeds were sown (soil + 500 g arbuscular mycorrhizal fungi) for 21 days, then transplanted to the cultivated land (Akanvou et al., 2007). Legumes (peanuts, mung beans, soybeans) were intercropped after the upland rice plants were 3 weeks after planting (WAP) (Arifuddin et al., 2019). Before planting, legumes were coated with 10 g/kg legin (Arifuddin et al., 2019). Legin (Legume Inoculant) is a biofertilizer in the form of an inoculant containing *Rhizobium* bacteria designed to colonize the roots of leguminous plants. Irrigation was carried out every 2–3 days according to weather conditions. Seedling of rice and legumes was transplanted by immersing 2 seeds in each planting hole.

SP-36 fertilizer 75 kg.ha^{-1} (36% P_2O_5, 5% S) and KCl 50 kg.ha^{-1} (60% K_2O) have been given 3 days before planting, a urea fertilizer dose of 200 kg.ha^{-1} (46% N) was given when the rice plant was 7 and 21 Days After Planting (DAP) or before flowering, with each ½ dose, namely 100 kg.ha^{-1} (46 kg N.ha^{-1}) by being buried between rows of plants (rows). Base fertilizer has been given when transplanting legumes with a dose of 25 kg.ha^{-1} Urea + 100 kg.ha^{-1} SP-36 (2/3 dose) + 100 kg.ha^{-1} KCl (2/3 dose) (IP2TP, 2000). When the legume plants were 4 weeks (28 Days After Sowing), follow-up fertilizer was given as much as 25 kg.ha^{-1} Urea + 100 kg.ha^{-1} SP-36 (1/3 dose) + 100 kg.ha^{-1} KCl (1/3 dose) by burying it between rows of plants (rows) 5 cm from the planting hole. Dolomite has been given at a dose of 400 kg.ha^{-1} when the legume plant is 1 WAP and 3 WAP (each ½ dose by burying it in the hole at a distance of 7 cm from the planting hole. Upland rice was harvested after 13 WAP, and legume was harvested after 11 WAP for mung beans and soybeans, 12 WAP for peanuts. Depending on plant conditions, the intercropping of upland rice with legumes on the same land lasted for ±10 weeks.

The number of samples used for each experimental unit was five plants. The research variables observed were plant growth components, plant yields, and the land use efficiency of intercropping systems compared to monocropping. Plant height (cm) was measured from the base of the plant to the tip of the tiller of the plant using a ruler at 35 and 56 DAP (Cavite et al., 2021). The number of tillers and the number of leaves were counted manually at 35 and 56 DAP by physically separating them and counting the number of tillers and the number of leaves on each plant, ensuring that each plant was a separate unit. SPAD lead greenness index was measured at 70 DAP using a portable chlorophyll meter that inserts a leaf into a sensor at the midpoint of the topmost fully developed leaf, and 5 representative plants were randomly selected for the measurements in each experimental unit (Yue et al., 2020). Dry weight of roots, dry weight of shoots, and total dry weight ($g.plant^{-1}$) were oven dried at 80 °C until they reached a constant weight, then the dry weight was measured using a digital scale at 56 DAP. The number of filled grains and the number of empty grains per plant were calculated at harvest time using a seed counter. The weight of filled grains and the weight of empty grains were calculated based on the dry grain weight at harvest. Rice productivity was measured as yield per hectare ($q.ha^{-1}$), calculated from the total harvested rice grain for a given area (Gollin & Udry, 2021).

Because intercropping systems are diverse and complex, evaluation is challenging (Khanal et al., 2021). Land equivalent ratio (LER) is the land area required by crops in a sole cropping system to produce the same yield as crops in a dual cropping system, compared to the land area required by component crops to produce the same yield for sole cropping (Karunarathna & Maduwanthi, 2022). Crop biomass yield per unit area is typically used to calculate the land equivalent ratio (LER), which is a commonly used indicator of the yield advantage of multi-crop farms over monocropping (Deb & Dutta, 2022). A multifunctional LER_M that uses LER_P for provisioning and LER_R for regulating services. The productivity LERP component was determined using the following method for systems with only two components, as simulated here (Khasanah et al., 2020). A LER of more than 1.0 indicates a positive intercropping advantage and higher interspecific facilitation compared to interspecific competition. The land equivalent ratio can be calculated by using the following formula.

$$LER = LER\ a + LER\ b = \frac{Y_{ab}}{Y_{aa}} + \frac{Y_{ba}}{Y_{bb}}$$

Where Yab (the yields of rice in intercropped rice–legumes) and Yba (the yields of legumes intercropped with rice–legumes) were the LER of two components in intercropping, and Yaa and Ybb were those in sole cropping of rice and sole cropping legumes, respectively.

The Competitive Ratio (CR) is an index used to indicate the degree of dominance or relative competitiveness of one species over another in an intercropping system; the higher a plant's CR value, the stronger its competitiveness compared to its companion plant (Willey & Rao, 1980). The competitiveness ratio is a better measure of plant competitiveness than relative crowding coefficient (k) and aggressivity level (A) (Karunarathna & Maduwanthi, 2022). The Competitive ratio can be calculated by using the following formula.

$$CRa = \left(\frac{LER_a}{LER_b}\right) x \left(\frac{Z_{ba}}{Z_{ab}}\right)$$

$$CRb = \left(\frac{LER_b}{LER_a}\right) x \left(\frac{Z_{ab}}{Z_{ba}}\right)$$

Where LERa (Land Equivalent Ratio of rice), LERb (Land Equivalent Ratio of legumes, Z (sown proportion of rice in intercropping), Z (sown proportion of legumes in intercropping). Crops can be grown in association if the Cra value is less than 1. The opposite is matching crop b.

Data Analysis. Compiled data was analyzed using analysis of variance (ANOVA), followed by the Post Hoc Tukey's HSD (honestly significant difference) Test at the α level of 5%. A t-test analysis was used to determine the difference between the means of cropping patterns: intercropping and sole cropping (Mishra et al., 2019). The statistical analysis was performed using Rstudio and Microsoft Excel.

Results and Discussion

Rainfall distribution. The rainfall situation of tested location was depictd in Figure 2, with the monthly rainfall (mm) from April to August 2025 is 306, 325, 122, 44, and 44 mm, respectively. Based on those rainfall distribution, the study area has two consecutive wet months (April–May) followed by one humid month (June) and two dry months (July–August), so according to Oldeman's classification, it is classified as climate type D2. This is also supported by data from the past 30 years (1991–2021) and 5 years (2018–2022) in the Banyumas Regency Regional Regulation. Climate type D2 is suitable for one planting of paddy or one palawija crop, depending on the supply of irrigation water (Aldiansyah & Risna, 2023; Oldeman, 1980). The study area is classified as Oldeman D2 and is suitable and relevant for the application of rice–legume intercropping. Water availability during the study was sufficient for the vegetative phase, but there was a deficit in the final phase. This risk can be mitigated by selecting the right planting time and applying intercropping with drought-tolerant legumes (Khatun et al., 2021).

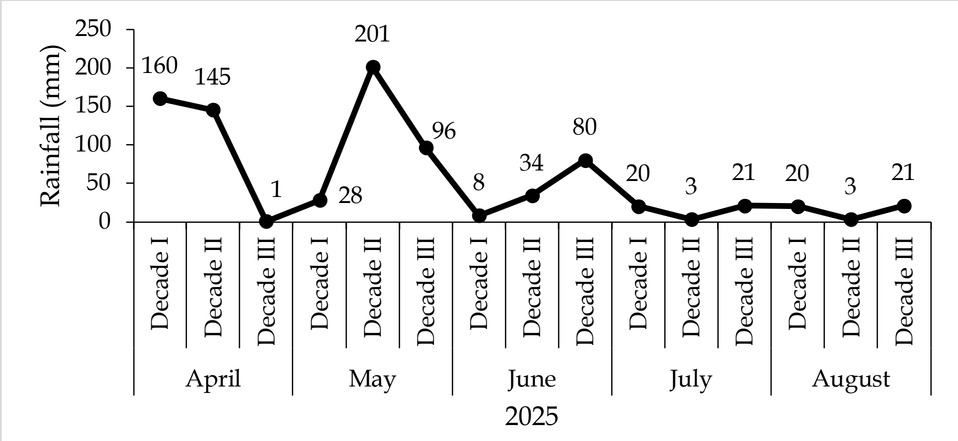


Figure 2. Rainfall distribution at the research site from April to August 2025 (BPSB, 2025)

Cereal–legume intercropping increases biomass, yield per unit area, and has a land equivalent ratio (LER) >1, with a progressively more pronounced yield advantage over sole cropping under conditions of progressive drought (Wang et al., 2026). This is particularly relevant for D2, as its rainfall pattern is not as consistent as that of wetter regions; therefore, a system that is more resilient to rainfall fluctuations will be more secure both agronomically and economically (Liu et al., 2025). Drought-tolerant legumes included peanuts, mung beans, and soybeans. Peanuts and mung beans are classified as drought-tolerant legumes, with peanuts having the highest level of tolerance, soybeans are classified as legumes with lower drought tolerance, especially in the generative phase, making them less adaptive to limited water availability compared to the other two legumes (Luo & Lin, 2025). The planting time for upland rice was the third week of April, and it was harvested in the fourth week of July. Legumes were planted in the second week of May and harvested in the fourth week of July (green beans and soybeans) and the first week of August (peanuts).

Plant height and number of tillers. The ability of a species to compete for light is significantly influenced by plant height, which is a central component of the plant's ecological strategy. Additionally, plant height is strongly correlated with life span, seed mass, and time to maturity, above-ground biomass, plant fitness, and leaf photosynthesis (Wang et al., 2019). There was no interaction effect on plant height among the treatment factors tested. The result showed that the plant height of upland rice in intercropping was significantly higher than in sole cropping at 35 and 56 days after planting (DAP) of 60.58 cm and 77.18 cm, respectively (Table 1). This may be due to the symbiosis that occurs between legumes that can fix N with rice. Plants can release root exudates, and organic compounds become signals that attract microorganisms to approach them so that they form a symbiotic relationship (plants – rhizosphere bacteria–plants). Root exudates usually create a nutrient-rich rhizosphere micro-environment, where microbial activity is stimulated. Root exudates consist of various primary and secondary compounds, including carbohydrates, amino acids, organic acids, phenolics, flavonoids, and auxins (Wu et al., 2023). However, the treatment of intercropping proportions and the type of legume used did not significantly affect plant height (Table 1).

The tiller number per unit area is a critical agronomic factor in determining yield. Consequently, real-time methods involving manual counting are considered a precise and efficient method for obtaining the tiller number in field conditions can be beneficial in establishing a reasonable group density (Fang et al., 2020). There was no interaction effect on the number of tillers per plant among the treatment factors tested. The variable number of tillers per clump was not affected by the treatment of intercropping proportions, type of legume, or planting pattern used. This may be due to the vegetative phase of tillering being influenced by factors such as light, temperature (Wang et al., 2024), and the application of nitrogen fertilizer (Kalaitzidis et al., 2025), which, in this case, both in intercropping and sole cropping treatments, all received the same fertilizer treatment. According to the results (Table 1), the number of tillers per plant at 56 DAP reached 27.07 for intercropping and 27.83 for sole cropping, while according to the results of the study (Idaryani et al., 2017), the number of tillers reached 20.12 at harvest. According to the variety description, the number of productive tillers of Situ Badegendit upland rice was 12–13 stems per clump (Simanulang, 2008). The proportion of rice-legume and the types of legume did not inhibit the growth of rice intercropped at 35 and 56 DAP. Rice habitus in the period 35–56 DAP has not had much influence or competition with the legume. It has different nutrient requirements, so there is no difference in the intercropping or sole cropping system.

Number of leaves, number of panicles, and SPAD leaf greenness. The leaf is the organ that is essential for the production of nutrients and photosynthesis in plants. Consequently, the quantity of leaves is one of the primary indicators used to describe the development and growth of a canopy, flowering time, and yield potential (Lu et al., 2021). The number of panicles per plant is the main trait that determines the grain yield in rice (Gunasekaran et al., 2023). There was no interaction effect on the number of leaves and number of panicles among the treatment factors tested. The number of leaves and panicles was not affected by the intercropping proportion, legume type, and cropping system used (Table 2). This may be because during the 35–56 DAP, the main rice crop was not yet competing for light and water with the intercropping (legume) crop. Rice panicles generally begin to form at 40–45 days (panicle initiation) and then continue to develop until the panicles emerge and enter the flowering stage at 50–55 days. The number of tillers formed is an important stage in determining the number of panicles (Wang et al., 2024). The number of tillers (Table 1) and the number of panicles (Table 2) are both the same and do not significantly affect the treatment given. The more tillers that form, the greater the chance of producing offspring that will yield panicles. The number of panicles of 56 DAP Situ Bagendit upland rice was 16.12 in intercropping and 11.67 in sole cropping, as indicated by the data (Table 2). Meanwhile, according to the results of the study (Aziz et al., 2010), the number of panicles reached 37.2 at harvest. The number of productive tillers is part of the number of panicles per clump. The number of panicles per clump is one of the most decisive factors in determining yield, because the more panicles produced per clump, the greater the amount of grain produced, thereby increasing the potential for higher yields (Sution & Nurdin, 2015).

Table 1. Plant height and number of tillers at 35 and 56 DAP as affected by different proportions of upland rice intercropped with various legume types

Treatments	Plant height (cm)		Number of tillers per plant	
	35 DAP	56 DAP	35 DAP	56 DAP
Cropping patterns				
Intercropping	60.58 a	77.18 a	23.87 a	27.07 a
Sole cropping	55.97 b	72.39 b	21.17 a	27.83 a
Intercropped Rice-Legume Proportion				
1:1	61.68 a	76.35 a	24.20 a	27.91 a
2:1	60.78 a	78.06 a	24.33 a	27.16 a
3:1	59.28 a	77.12 a	23.07 a	26.13 a
CV (%)	7.71	3.02	14.93	15.86
Types of Legumes				
Peanuts	61.49 a	76.89 a	24.80 a	27.67 a
Mung beans	59.88 a	77.17 a	22.38 a	24.80 a
Soybeans	60.37 a	77.47 a	24.42 a	28.73 a
CV (%)	5.21	4.46	14.09	14.44
Interaction	-	-	-	-

Note: DAP (Days After Planting), (-) no interaction, CV (Coefficient of Variance). Cropping patterns were analyzed using the t-test. The average number of rice-legume intercropping followed by the same letter in each column indicates no significant difference in the Tukey HSD test at α = 95%.

Table 2. Number of leaves, number of panicles, and SPAD leaf greenness as affected by different proportions of upland rice intercropped with various legume types

Treatment	Number of leaves		Number of panicles*	SPAD leaf greenness index
	35 DAP	56 DAP	56 DAP	70 DAP
Cropping patterns				
Intercropping	86.10 a	112.30 a	16.12 a	38.39 b
Sole cropping	73.92 a	120.67 a	11.67 a	40.83 a
Intercropped Rice-Legume Proportion				
1:1	88.47 a	116.51 a	15.70 a	38.36 a
2:1	87.60 a	113.87 a	15.11 a	38.32 a
3:1	82.22 a	106.51 a	17.56 a	38.50 a
CV (%)	12.29	17.91	35,82	3.41
Types of Legumes				
Peanuts	89.47 a	115.09 a	16 a	38.06 a
Mung beans	79.53 a	104.22 a	16.93 a	38.15 a
Soybeans	89.29 a	117.58 a	15.44 a	38.96 a
CV (%)	12.96	16.24	27.72	3.58
Interaction	-	-	-	-

Note: DAP (Days After Planting), (-) no interaction, CV (Coefficient of Variance). Cropping patterns were analyzed using the t-test. The average number of rice-legume intercropping followed by the same letter in each column indicates no significant difference in the Tukey HSD test at α = 95%.

The soil and plant analyzer development (SPAD) value is an important indicator affecting rice yield and quality. The SPAD leaf greenness index is frequently used to describe a plant's concentration of nitrogen or chlorophyll (Zhang et al., 2024). There was no interaction effect on SPAD leaf greenness index among the treatment factors tested. The greenness index variable of SPAD upland rice leaves at 70 DAP in the sole cropping system is significantly higher than in the intercropping system (Table 2). The greenness of the leaves is closely related to the chlorophyll content in a plant tissue (Yue et al., 2025). Chlorophyll formation is very dependent on the availability of nitrogen, where the addition of nitrogen to a certain level will be followed by an increase in chlorophyll concentration (Elsayed et al., 2023). Fertilizer applied to sole cropping land is more focused on being absorbed by rice, while in intercropping land, the nutrients are divided for the needs of two plants (rice and legumes) with a denser plant population. In addition, rice aged at 70 DAP, the legume intercropped plants have begun to enter the maximum vegetative phase, which indicates that the plants need more nutrients to prepare for flowering. In intercropping, legumes supply nitrogen to other plants through direct transfer via root systems, tilling into the soil, the breakdown of their roots, and nitrogen transfer facilitated by plant-associated mycorrhizae (Thilakarathna et al., 2016). This happens all during the growing season, but when the legume is cut off and the plant matter decomposes, a large quantity of nitrogen is released into the soil, allowing nearby crops to use it. Additionally, nitrogen from the decomposition of legume residues can be used to restore soil fertility; this primarily depends on how the residues are used-burned, completely removed from the field, or incorporated, which is more beneficial (Kebede, 2021). Although legumes can fix nitrogen, this process is more beneficial for subsequent crops rather than directly for the rice that is currently growing. Therefore, chlorophyll content (as reflected by SPAD) is higher in sole croppings.

Root dry weight, shoot dry weight, and total dry weight of rice. An irreversible increase in size, plant growth is commonly referred to as net primary production (Hilty et al., 2021). Dry weight is measured when the amount of plant material has been dried to a constant moisture content (Turnage, 2022). The results of the study showed that the intercropping system produced root dry weight, shoot dry weight, and total dry weight of rice that were not significantly different from those of upland rice in a sole cropping system (Table 3). There was no interaction effect on the root dry weight, shoot dry weight, and total dry weight of rice among the treatment factors tested. Higher biomass values were a good indicator of the plant's potential to produce large yields of grain (Xi et al., 2024). There were no differences between sole cropping and intercropping treatments at various proportions and types of legumes, indicating that the main rice crop can adapt to normal conditions as well as in dense populations. The root systems of rice and legumes differ in depth, so root complementarity likely reduced direct competition for belowground resources (Homulle et al., 2022). As a result, total biomass accumulation remains comparable. Based on the total dry weight data of rice (Table 3), the total dry weight of intercropped rice was 42.51 g.plant^{-1}, while that of sole cropping was 36.36 g.plant^{-1}. According to Meirelles et al. (2024), without influencing grain yield, upland rice interplanted with *C. breviflora* (legume) demonstrated a greater overall dry mass production than sole rice.

Number of filled grains, number of empty grains, weight of filled grains, weight of empty grains, and productivity of rice. The yield components of rice are the number of filled grains, the number of empty grains, the weight of filled grains, weight of empty grains. A mature rice grain that is full of nutrients and fully developed as a result of the grain filling stage is called a filled grain (Kumar et al., 2020). The endosperm, the primary component of the grain, is where proteins, lipids, and carbohydrates are created and accumulated during this process, affecting the grain's weight, yield, and quality (Xu et al., 2021). There was no interaction effect on the number of filled grains, number of empty grains, weight of filled grains, weight of empty grains, and productivity of rice among the treatment factors tested. The study showed that the number of filled grains and the weight of filled grains did not significantly affect the treatment of intercropping proportion, legume type, and cropping system used (Table 4). Meanwhile, the sole cropping treatment significantly affected the number of empty rice grains by 574.42 and in the intercropping system by 239.56. This further impacted the weight of empty grains, which was found to be significant in the sole cropping system (3.40 g.plant^{-1}) while in the intercropping system, it was 1.29 g.plant^{-1} (Table 4). This shows that in a sole cropping system with a relatively free planting area without competition, large amounts of grain were produced. However, the large number of empty grains found in the sole cropping system can be caused by the inability of the plants to fill the rice grains optimally. The rice spikelet may be successfully pollinated and fertile, but the grain filling was not optimal, so the resulting grain was not full (Parida et al., 2022).

Table 3. Root dry weight of roots, shoot dry weight, and total dry weight of rice plant as affected by different proportions of upland rice intercropped with various legume types

Treatment	Root dry weight (g.plant^{-1})*	Shoot weight (g.plant^{-1})*	Total dry weight (g.plant^{-1})*
	56 DAP	56 DAP	56 DAP
Cropping patterns			
Intercropping	5.17 a	36.96 a	42.51 a
Sole cropping	3.20 a	33.16 a	36.36 a
Intercropped Rice-Legume Proportion			
1:1	5.01 a	36.31 a	42.46 a
2:1	4.85 a	35.45 a	40.29 a
3:1	5.65 a	39.13 a	44.77 a
CV (%)	38.27	16.85	20.09
Types of Legumes			
Peanuts	5.07 a	37.46 a	42.53 a
Mung beans	5.64 a	39.94 a	45.07 a
Soybeans	4.79 a	33.48 a	39.92 a
CV (%)	21.48	13.19	13.21
Interaction	-	-	-

Note: DAP (Days After Planting), (-) no interaction, CV (Coefficient of Variance). Cropping patterns were analyzed using the t-test. The average number of rice-legume intercropping followed by the same letter in each column indicates no significant difference in the Tukey HSD test at α = 95%. (*) sign: the CV is the result of data transformation.

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Table 4. Yield component of rice plant as affected by different proportions of upland rice intercropped with various legume types

Treatment	Number of filled grains (plant^{-1})*	Number of empty grains (plant^{-1})*	Weight of filled grains (g.plant^{-1})*	Weight of empty grains (g.plant^{-1})*	Productivity of Rice (q.ha^{-1})*
Cropping patterns					
Intercropping	155.14 a	239.56 b	3.34 a	1.29 b	6.54 b
Sole cropping	191.17 a	574.42 a	4.89 a	3.40 a	13.39 a
Intercropped Rice-Legume Proportion					
1:1	151.62 a	226.53 a	3.30 a	1.19 a	6.97 a
2:1	171.24 a	255.49 a	3.62 a	1.39 a	7.17 a
3:1	142.56 a	236.64 a	3.11 a	1.28 a	5.48 a
CV (%)	22.52	18.78	19.63	22.30	20.65
Types of Legumes					
Peanuts	160.56 a	218.16 a	3.42 a	1.12 a	6.57 a
Mung beans	137.18 a	240.09 a	2.84 a	1.35 a	6.65 a
Soybeans	167.69 a	260.42 a	3.76 a	1.38 a	6.39 a
CV (%)	28.74	19.83	32.79	20.02	21.89
Interaction	-	-	-	-	-

Note: (-) no interaction. Cropping patterns were analyzed using the t-test. The average number of rice-legume intercropping followed by the same letter in each column indicates no significant difference in the Tukey HSD test at α = 95%. (*) sign: the CV is the result of data transformation.

Yield per hectare or productivity is a variable measured based on the dry grain yield. Yield is an important agronomic variable that serves as an indicator of whether a superior variety will be accepted or adopted by farmers (Sution & Nurdin, 2015). In intercropping, competition for water and nutrients during the grain filling phase reduced rice productivity. However, the amount of empty grains was lower because assimilate distribution was more efficient for the grains that are formed (sink limitation). Sole cropping excels in total yield because all resources are focused solely on rice. The potential yield of a variety can only be achieved if it is planted in growing conditions that are suitable for that variety. Differences in weather, water management, and soil type result in varying yields. The use of superior varieties in efforts to increase production plays a very important role. In addition, the yield potential of a particular variety cannot be separated from its level of adaptation and stability in a given growing environment research (Idaryani et al., 2017).

Land Equivalent Ratio (LER) and Competitive Ratio. One of the key variables in assessing the effectiveness of an intercropping system is the land equivalent ratio (LER) value. The LER indicates how much sole cropping land is needed to produce the same amount of yield as 1 hectare of intercropped land. A LER value greater than 1 indicates that land productivity (intercropping) is more efficient than sole cropping; conversely, if the LER value <1, then productivity is low (Dharmawangsa et al., 2020). The results showed that the best total LER value was obtained with an intercropping proportion of 2:1, at 1.68, indicating that intercropping provided 68% greater benefits than sole cropping. The second-highest LER was a proportion of 1:1 (1.25), and the lowest was a proportion of 3:1 with an LER of 1.20 (Table 5). In addition, the best LER of legumes was obtained in intercropping with peanuts, with a LER value of 2.13, followed by soybeans (0.35), and the lowest was mung beans with an LER of 0.17. Peanuts were highly suitable for intercropping with upland rice, whereas soybeans and mung beans have an LER <1, meaning that these two types of legumes are not land-efficient. Intercropping systems with legumes not only offer increased productivity through commodity diversification but also have beneficial effects on soil texture, microbial diversity, water retention, plant growth, and crop yields (Kokkini et al., 2025).

LER is a derived variable, not a direct variable such as plant height or rice yield. LER combines two yield components (rice + legumes) simultaneously. Although there were no significant differences in rice yield among the treatments, the contribution of legumes can vary greatly. Based on the results

(Table 4), rice yields did not differ significantly among treatments, but legume LERs did differ, resulting a difference in total LER. LER is a system-level index of intercropping performance because it integrates the relative yields of both component species against their respective sole crops, thereby reflecting land-use efficiency and the net outcome of complementarity and competition within the system. Therefore, LER may show significant treatment differences even when individual component responses are not statistically significant (Mead & Willey, 1980).

Intercropping upland rice with legumes increased land efficiency, growth variables, and plant biomass, which were relatively comparable to sole cropping. However, pure upland rice yields (grain productivity) remain higher in sole cropping because there was no competition with legumes. Legumes serve a long-term function in soil fertility (N-fixation) and crop diversification, making this system suitable for upland areas at risk. The proportions of 1:1, 2:1, and 3:1 were still within a relatively narrow range. The highest total LER at a ratio of 2:1 reflects conditions where the benefits of intercropping (resource complementarity and nitrogen fixation) outweigh the disadvantages of competition. This ratio represents the optimum point between the dominance of upland rice as the main crop and the contribution of legumes as companion crops, especially in agroclimatic conditions with limited water availability.

The competitive ratio (CR) in intercropping indicates how much more competitive one plant component is than another (Willey & Rao, 1980). Intercropping evaluation indices, including competitive ratio, assess different aspects of intercropping system performance, and intercropping yields are influenced by total planting density as well as the proportion of crops in the intercropping system (Zustovi et al., 2024). Based on the crop proportion, all competitive ratios were greater than 1, indicating that both crops were actively competing. At a proportion of 2:1 and 3:1, the legume was more competitive than the upland rice; however, even when the legume was dominant, the intercropping system remained efficient (high LER). The competitive ratio varies with planting density, so the competitive ratio is also useful for evaluating the design of intercropping proportions or densities (Y. Wang et al., 2024).

Based on the competitive ratio (CR) and land equivalent ratio (LER) values, peanuts exhibit a very high level of competitiveness in an intercropping system with upland rice. Nevertheless, this combination yields the highest total LER value of 2.63; thus, it can be considered the most profitable and ideal combination for the implementation of intercropping. Conversely, for mung beans, a CR value <1 indicated that this crop was outcompeted by upland rice, and this condition aligns with the low LER value, the combination considered less suitable for intercropping systems. As for soybeans, the CR value indicates relatively more balanced competition; however, because the LER value was <1, the rice–soybean intercropping system is still considered less efficient in terms of land use. In intercropping, there is indeed competition for light, water, and nutrients, but at the same time, two species can also complement each other through differences in canopy height, root depth, nutrient uptake timing, or the ability of legumes to fix nitrogen (Neamatollahi et al., 2013). System efficiency often arises due to resource partitioning and facilitation, not because competition is eliminated. Therefore, even if one crop appears to be "dominant" in terms of CR, the system can still be more productive overall, resulting in an LER >1, indicating greater land-use efficiency (Wang et al., 2024).

Table 5. The land equivalent ratio and competitive ratio of intercropped rice-legume

Intercropped Rice-Legume	Land Equivalent Ratio			Competitive Ratio	
	Rice	Legume	Total	Rice	Legume
Intercropped Rice-Legume Proportion					
1:1	0.53	0.72	1.25	1.70	1.54
2:1	0.54	1.15	1.68	1.82	3.09
3:1	0.41	0.79	1.20	2.37	2.79
HSD (P≤0.05)	0.24	0.32	0.07	1.01	0.93
Types of Legumes					
Peanuts	0.50	2.13	2.63	1.82	5.22
Mung beans	0.50	0.17	0.67	2.94	0.47
Soybeans	0.48	0.35	0.83	1.13	1.74
HSD (P≤0.05)	0.19	0.73	0.63	0.66	1.11

Intercropping rice and peanuts were more productive than other legumes. LER data showed that peanuts contribute more than mung beans or soybeans. The weight of peanut seeds per plant in intercropping and sole cropping did not differ significantly (data unpublished). From rainfall data (Figure 2), it is known that during the generative phase of the plants, July and August were dry months, indicating drought. Peanuts were known as the most tolerant legume to limited water availability because they have a strong and deep tap-root system, relatively low transpiration rates, and the ability to adapt to light-textured soils (Zhen et al., 2024). These plants were still able to produced well in relatively low rainfall, with critical phases mainly during gynophore formation and pod filling (Dong et al., 2024).

Mung beans have moderate to high drought tolerance, mainly because of their short growing season, which allows them to complete their life cycle before water stress reaches severe levels (drought escape mechanism) (Wenham et al., 2019). However, mung beans remain sensitive to water deficits during the flowering and pod filling stages (Singh et al., 2021). Meanwhile, soybeans are the most drought-sensitive legume of the three, because their root system is relatively shallow and their water requirements are higher, especially during the generative phase (Wang et al., 2022). Water deficits during the flowering and seed filling phases in soybeans can cause significant yield losses, so this crop requires a more stable water supply than peanuts and mung beans (Aziez & Prasetyo, 2022).

There was no interaction between planting proportions and legume types was likely due to the high degree of niche complementarity between rice and legumes, both temporally and spatially. The absence of interaction between the two factors indicates that the system's response did not depend on specific treatment combinations. Legumes were planted 3 weeks after rice, so their resource-demand phases did not fully overlap. Furthermore, differences in root system characteristics and nutrient utilization patterns result in relatively low direct competition between crops. Consequently, interspecific interactions were relatively weak, and the growth and yield responses of upland rice remained consistent across different planting proportions and legume types.

The differences in proportions remained within a non-extreme range, so the system was not sufficiently disrupted to trigger interactions, and the variability among treatments was small (Zustovi et al., 2024). The selected legume species share the same ecological function, namely, nitrogen fixation (Kebede, 2021). Although the selected legume species differ in root architecture, drought response, and crop duration, these species-specific traits may not have been fully expressed under the present experimental conditions (Gelaye et al., 2025; Islam et al., 2021; Singh et al., 2021; X. Wang et al., 2022). Water availability was still adequate during the vegetative stage, the legumes were introduced three weeks after rice, and the tested planting proportions were within a relatively narrow range; therefore, interspecific competition remained limited, and treatment variability was small (Meirelles et al., 2024).

Despite the non-significant effects on rice yield components, the intercropping system of upland rice with peanut remains agronomically advantageous. This is evidenced by the high land equivalent ratio (LER), which reached 2.63 in the rice–peanut combination, indicating substantial land-use efficiency compared to monocropping. Similarly, the 2:1 planting proportion resulted in the highest LER (1.68), suggesting an optimal balance between rice dominance and legume contribution. Although the competitive ratio (CR) indicated that peanut was more dominant in resource utilization, this did not negatively affect the overall system performance; instead, it contributed to higher total land productivity. From a farmer's perspective, such dominance is acceptable as long as the combined yield and land efficiency increase. This interpretation is supported by recent studies showing that cereal–legume intercropping can stabilize yield and economic returns under variable rainfall, while farm-level analyses also demonstrate that legume–cereal intercropping can improve profitability through better land use and reduced input costs (Wang et al., 2026). In addition, peanut's greater adaptability to dry conditions, particularly under late-season water limitation, makes it a more reliable companion crop than mung bean or soybean. Importantly, rice growth and yield were not significantly reduced across treatments, indicating that intercropping provides additional output without compromising the main crop. Therefore, the adoption of upland rice–peanut intercropping can be justified not only by ecological complementarity but also by its capacity to enhance land-use efficiency, diversify production, and reduce farming risks under variable environmental conditions.

Conclusion

Differences in the proportion of upland rice–legume intercropping and legume types did not significantly affect the growth and yield of upland rice. However, rice–legume intercropping improved land-use efficiency, particularly at the 2:1 proportion with an LER >1 (1.68) and in the upland rice–peanut combination, which produced the highest LER >1 (2.63). Although the competitive ratio showed that some legumes, especially peanuts, were more competitive than rice, this dominance was not detrimental because the intercropping system still achieved an LER greater than 1. In contrast, the mung bean and soybean combinations were less efficient and are therefore not recommended.

Acknowledgments

The Authors gratefully acknowledge the 2025 BLU research grants by the Institute of Research and Community Service (LPPM) of Jenderal Soedirman University, under the Competency Enhancement Research (RPK) scheme and grant number (14.519/UN23.34/PT.01/V/2025).

References

Afonso P, Castro I, Couto P, Leal F, Carnide V, Rosa E, Carvalho M. 2025. Root Phenotyping : A Contribution to Understanding Drought Stress Resilience in Grain Legumes. *Agronomy*, *15*(4), 1–15.

Akanvou RK, Becker M, Bastiaans L, Kropff MJ. 2007. Morpho–physiological characteristics of Cover crops for analysis of upland rice production in relay intercropping systems. *Sciences & Nature*, *4*(2), 205–216.

Aldiansyah S, Risna. 2023. Mapping of oldeman agro-climatic zone based on climate hazards group infrared precipitation with station database in Southeast Sulawesi. *Ecotrophic*, *17*(2), 174–187.

Amanullah F, Ropalia, Lestari T. 2022. Budidaya kacang hijau ramah lingkungan. *Seminar Nasional Penelitian Dan Pengabdian Pada Masyarakat 2022*, 57–62.

Andrews DJ, Kassam AH. 1976. The Importance of Multiple Cropping in Increasing World Food Supplies. In *American Society of Agronomy* (Vol. 27). https://doi.org/10.2134/asaspecpub27.c1

Arifuddin A, Wangiyana W, Aryana I. 2019. Effect of relay-planting several legume species at various ages of rice on growth and yield of red rice grown together with legume crops under aerobic irrigation system. *International Journal of Environment, Agriculture and Biotechnology*, *4*(6), 1704–1710.

Assefa E, Bitew Y. 2023. Enhancing the land use efficiency of low-land rice (*Oryza sativa* L.)—Grass pea (*Lathyrus sativus* L.) additive series relay intercropping in North-Western Ethiopia: A farmer's indigenous knowledge. *PLoS ONE*, *18*(7 July), 1–17. https://doi.org/10.1371/journal.pone.0281410

Aziez AF, Prasetyo A. 2022. Root growth response of soybean (*Glycine max* L.) under water deficit. *Journal of Biodiversity and Biotechnology*, *2*(2), 63–69.

Aziz A, Basri AB, Nasir A, Pesireron M. 2010. Uji multilokasi 6 varietas padi gogo di Provinsi NAD. *Prosiding Seminar Nasional: Mewujudkan Kedaulatan Pangan Pada Lahan*, *27*, 338–345.

Beets WC. 2019. *Multiple cropping and tropical farming systems*. CRC Press.

Benu FL, Lawa Y, Neolaka YAB. 2023. Mini Review: Peran biofertilizer pada pertanian lahan kering. *Jurnal Beta Kimia*, *3*(1), 40–49. https://doi.org/10.35508/jbk.v3i1.11656

BPS. 2023. *Harvested Area, Production and Yield Rate of Dryland Paddy, 2023*. BPS Kabupaten Banyumas. https://banyumaskab.bps.go.id/id/statistics-table/1/NjM1IzE%3D/luas-panen-produksi-dan-rata-rata-produksi-padi-ladang-tahun-2023.html

BPS Kabupaten Banyumas. 2026. *Kabupaten Banyumas Dalam Angka 2026 Volume 51*. https://banyumaskab.bps.go.id/id/publication/2026/02/27/4e4f855322bf9f80022acafb/kabupaten-banyumas-dalam-angka-2026.html

Bukovsky-Reyes S, Isaac ME, Blesh J. 2019. Effects of intercropping and soil properties on root functional traits of cover crops. *Agriculture, Ecosystems and Environment*, *285*(106614), 1–10. https://doi.org/10.1016/j.agee.2019.106614

Cavite HJM, Mactal AG, Evangelista EV, Cruz JA. 2021. Growth and yield response of upland rice to application of plant growth-promoting rhizobacteria. *Journal of Plant Growth Regulation*, *40*(2), 494–508. https://doi.org/10.1007/s00344-020-10114-3

Deb D, Dutta S. 2022. The robustness of land equivalent ratio as a measure of yield advantage of multi-crop systems over monocultures. *Experimental Results*, *3*, 1–13. https://doi.org/10.1017/exp.2021.33

Destiningsih R. 2016. Analisis Komoditas Unggulan Pangan Kabupaten Banyumas. *Jurnal REP*, *1*(1), 35–48. https://doi.org/10.31002/rep.v1i1.51

Dharmawangsa L, Nujanah U, Pujiwati H, Setyowati N, Prasetyo P. 2020. Nilai kesetaraan lahan dan hasil jagung manis tumpangsari dengan kacang-kacangan di pertanian organik. *Seminar Nasional Lahan Suboptimal*, *1*, 224–236.

Dong J, Shen X, Li Q, Xue Z, Hou X, Miao H, Ning H. 2024. Irrigation and fertilization scheduling for peanut cultivation under mulched drip irrigation in a desert-oasis area. *Plants (Basel, Switzerland)*, *13*(1), 144. https://doi.org/10.3390/plants13010144

Elsayed S, El-Hendawy S, Elsherbiny O, Okasha AM, Elmetwalli AH, Elwakeel AE, Memon MS, Ibrahim MEM, Ibrahim HH. 2023. Estimating chlorophyll content, production, and quality of sugar beet under various nitrogen levels using machine learning models and novel spectral indices. *Agronomy*, *13*(11), 2743.

Fang Y. Qiu X, Guo T, Wang Y, Cheng T, Zhu Y, Chen Q, Cao W, Yao X, Niu Q, Hu Y, Gui L. 2020. An automatic method for counting wheat tiller number in the field with terrestrial LiDAR. *Plant Methods*, *16*(1), 1–14. https://doi.org/10.1186/s13007-020-00672-8

Feng, L, Raza MA, Chen Y, Khalid MH Bin, Meraj TA, Ahsan F, Fan Y, Du J, Wu X, Song C, Liu C, Bawa G, Zhang Z, Yuan S, Yang F,Yang W. 2019. Narrow-wide row planting pattern improves the light environment and seed yields of intercrop species in relay intercropping system. *PLoS ONE*, *14*(2), 1–19. https://doi.org/10.1371/journal.pone.0212885

Galo E. 2025. Performance of upland rice intercropped legumes and level of nitrogen fertilizer. *International Journal of Agriculture and Biosciences 2025*. *14*(4), 740–747.

Gelaye Y, Li J, Luo H. 2025. Journal of Genetic Engineering and Biotechnology Exploring the role of Peanut (Arachis hypogaea L .) root architecture in enhancing adaptation to climate change for sustainable agriculture and resilient crop production : A review. *Journal of Genetic Engineering and Biotechnology*, *23*(3), 1–12. https://doi.org/10.1016/j.jgeb.2025.100535

Gollin D, Udry C. 2021. Heterogeneity, measurement error, and misallocation: Evidence from african agriculture. *Journal of Political Economy*, *129*(1), 1–80. https://doi.org/10.1086/711369

Gunasekaran A, Seshadri G, Ramasamy S, Muthurajan R, Karuppasamy KS. 2023. Identification of newer stable genetic sources for high grain number per panicle and understanding the gene action for important panicle traits in rice. *Plants*, *12*(2), 1–18. https://doi.org/10.3390/plants12020250

Harsono A, Harnowo D, Ginting E, Elisabeth DAA. 2021. Soybean in Indonesia: current status, challenges and opportunities to achieve self-sufficiency. In J. C. Jimenez-Lopez & A. Clemente (Eds.), *Legumes Research - Volume 1*. IntechOpen. https://doi.org/10.5772/intechopen.101264

Hawalid H. 2019. Respon pertumbuhan dan produksi tanaman kacang tanah (*Arachis hypogaea* L.) pada pemberian takaran pupuk organik cair limbah tahu dan jarak tanam yang berbeda. *Klorofil*, *xiv*(2), 78–82.

Heryani N, Rejekiningrum P. 2020. Pengembangan pertanian lahan kering iklim kering melalui implementasi panca kelola lahan. *Jurnal Sumberdaya Lahan*, *13*(2), 63–71. https://doi.org/10.21082/jsdl.v13n2.2019.63-71

Hilty J, Muller B, Pantin F, Leuzinger S. 2021. Plant growth: the what, the how, and the why. *New Phytologist*, *232*(1), 25–41. https://doi.org/10.1111/nph.17610

Homulle Z, George, TS, Karley AJ. 2022. Root traits with team benefits : understanding belowground interactions in intercropping systems. *Plant and Soil*, *417*, 1–26. https://doi.org/10.1007/s11104-021-05165-8

Idaryani, Warda, Dahamarudin L. 2017. Karakter agronomis dan hasil beberapa varietas unggul padi pada lahan kering di Kabupaten Jeneponto. *Prosiding Seminar Nasional: Mewujudkan Kedaulatan Pangan Pada Lahan Sub Optimal Melalui Inovasi Teknologi Pertanian Spesifik Lokasi*, 202–207.

IP2TP. 2000. Budidaya Kacang Tanah di Lahan Kering. *Instalasi Penelitian dan Pengkajian Teknologi Pertanian Mataram, Lembar Informasi Pertanian (Liptan) IP2TP Mataram No. 09/Liptann/2000*, 2–4.

Iwuagwu C, Umechuruba C, Ononuju C, Nwogbaga A, Salaudeen M, Onejeme F. 2019. Effect of intercropping rice with maize and soybeans on disease incidence, severity and yield of rice in Abia State. *International Journal of Agricultural Science*. *4*, 23–28. http://iaras.org/iaras/journals/ijas

Jarrar H, El-Keblawy A, Ghenai C, Abhilash PC, Bundela AK, Abideen Z, Sheteiwy MS. 2023. Seed enhancement technologies for sustainable dryland restoration: Coating and scarification. *Science of The Total Environment*, *904*, 150–166. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.166150

Kalaitzidis A, Kadoglidou K, Mylonas I, Ghoghoberidze S, Ninou E, Katsantonis D. 2025. Investigating the impact of tillering on yield and yield-related traits in European rice cultivars. *Agriculture; Basel*, *15*(6).

Karunarathna B, Maduwanthi AKMRB. 2022. Competition indices used to evaluate the agronomic and monetary advantage in intercropping: A review. *AGRIEAST: Journal of Agricultural Sciences*, *16*(1), 25–40. https://orcid.org/0000-0002-3773-9402 25

Kebede E. 2021. Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. *Frontiers in Sustainable Food Systems*, *5*, 1–18. https://doi.org/10.3389/fsufs.2021.767998

Khanal U, Stott KJ, Armstrong R, Nuttall JG, Henry F, Christy BP, Mitchell M, Riffkin PA, Wallace AJ, Mccaskill M, Thayalakumaran T, Leary GJO. 2021. Intercropping — evaluating the advantages to broadacre systems. *Agriculture*, *11*(453), 1–20. https://doi.org/https://doi.org/10.3390/agriculture11050453

Khasanah N, van Noordwijk M, Slingerland M, Sofiyudin M, Stomph D, Migeon AF, Hairiah K. 2020. Oil palm agroforestry can achieve economic and environmental gains as indicated by multifunctional land equivalent ratios. *Frontiers in Sustainable Food Systems*, *3*(January), 1–13. https://doi.org/10.3389/fsufs.2019.00122

Khatun M, Sarkar S, Era FM, Islam AKMM, Anwar MP, Fahad S, Datta R, Islam AKMA. 2021. Drought stress in grain legumes: effects, tolerance mechanisms and management. *Agronomy*, *11*(12). https://doi.org/10.3390/agronomy11122374

Kokkini M, Gazoulis I, Danaskos M, Kontogeorgou V, Kanatas P, Travlos I. 2025. Enhancing ecosystem services in agriculture: the special role of legume intercropping. *Frontiers in Sustainable Food Systems*, *9*, 1547879.

Kumar A, Taparia M, Madapu A, Rajalakshmi P, Marathi B, Desai UB. 2020. Discrimination of filled and unfilled grains of rice panicles using thermal and RGB images. *Journal of Cereal Science*, *95*, 1–9. https://doi.org/10.1016/j.jcs.2020.103037

Liu H, Gao X, Li C, Cai Y, Song X, Zhao X. 2025. Intercropping increases plant water availability and water use efficiency: A synthesis. *Agriculture, Ecosystems & Environment*, *379*, 109360. https://doi.org/https://doi.org/10.1016/j.agee.2024.109360

Lu S, Song Z, Chen W, Qian T, Zhang Y, Chen M, Li G. 2021. Counting dense leaves under natural environments via an improved deep-learning-based object detection algorithm. *Agriculture (Switzerland)*, *11*(10), 1–16. https://doi.org/10.3390/agriculture11101003

Luo D, Lin H. 2025. Evaluation of drought-tolerant legume varieties under rainfed conditions. *Field Crop*, *8*(3), 126–138. https://doi.org/10.5376/fc.2025.08.0013

Mead R, Willey RW. 1980. The Concept of a 'Land Equivalent Ratio' and Advantages in Yields from Intercropping. Experimental Agriculture, 16(3), 217–228.

Meirelles FC, Cavalcante AG, Gonzaga AR, Coelho AP, van der Werf W, Bastiaans L, Arf O, Lemos LB. 2024. Relative sowing time and spatial arrangement in upland rice/legume intercropping systems. *International Journal of Plant Production*, *18*(2), 161–174. https://doi.org/10.1007/s42106-024-00294-3

Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. 2019. Application of student's t-test, analysis of variance, and covariance. *Annals of Cardiac Anaesthesia*, *22*(4), 407–411. https://doi.org/10.4103/aca.aca_94_19

Nandhini DU, Somasundaram E. 2020. Intercropping – A substantial component in sustainable organic agriculture. *Ind. J. Pure App. Biosci*. *8*(2), 133–143.

Neamatollahi E, Jahansuz MR, Mazaheri D, Bannayan M. 2013. Intercropping. In E. Lichtfouse (Ed.), *Sustainable Agriculture Reviews, vol 12.* (pp. 119–142). Springer Netherlands. https://doi.org/10.1007/978-94-007-5961-9_4

Oldeman RL. 1980. The agroclimatic classification of rice-growing environments in Indonesia. In *World Meteorological Organization; International Rice Research Institute: Proceedings of a symposium on the agrometeorology of the rice crop* (pp. 47–55).

Ozioma EM, Nwabunwanne OE, Ukaoba ME, Daniel OI. 2024. Substitutive effects of upland rice and soybean plant populations on the performance of the component crops in intercropping system. *System*, *12*(8), 13.

Paiman, Ardiyanta, Kusumastuti CT, Masulili A, Yussof SF. 2023. A review on the advantages of Jajar Legowo planting system in rice (*Oryza sativa* L .) cultivation. *Res. Crop*, *24*(3), 433–441. https://doi.org/10.31830/2348-7542.2023.ROC-966

Papong JR, Cagasan UA. 2020. Growth and yield performance of upland rice (*Oryza sativa* L . var . zambales) intercropped with mungbean *(Vigna radiata L .)* and peanut *(Arachis hypogaea L .)*.International Journal of Agriculture, Forestry and Life Sciences *1*, 34–41.

Parida AK, Sekhar S, Panda BB, Sahu G. 2022. Effect of Panicle Morphology on Grain Filling and Rice Yield : Genetic Control and Molecular Regulation. *Frontiers in Genetics*, *13*, 1–23. https://doi.org/10.3389/fgene.2022.876198

Peraturan Daerah Kabupaten Banyumas Nomor 12 Tahun 2024. 2024. *Rencana perlindungan dan pengelolaan lingkungan hidup TAHUN 2024-2054*. https://static.banyumaskab.go.id/jdih/file/Peraturan-Nomor-12-Tahun-2024-tentang-peraturan-daerah-kabupaten-banyumas-nomor-12-tahun-2024-tentang-rencana-perlindungan-dan-pengelolaan-lingkungan-hidup-tahun-2024-2054.pdf

Pokharel SS, Yu H, Fang W, Parajulee MN, Chen F. 2023. Intercropping cover crops for a vital ecosystem service: a review of the biocontrol of insect pests in tea agroecosystems. *Plants*, *12*(12), 1–14. https://doi.org/10.3390/plants12122361

Rambe SSM, Calista I, Wulandari TN, Saragih A. 2024. Budidaya Tanaman Padi Gogo Terstandar. In *Balai Penerapan Standar Instrumen Pertanian (BPSIP) Bengkulu*.

Raza MA, Cui L, Qin R, Yang F, Yang W. 2020. Strip-width determines competitive strengths and grain yields of intercrop species in relay intercropping system. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-78719-y

Raza MA, Din AMU, Zhiqi W, Gul H, Ur Rehman S, Bukhari B, Haider I, Rahman MHU, Liang X, Luo S, El Sabagh A, Qin R, Zhongming M. 2023. Spatial differences influence nitrogen uptake, grain yield, and land-use advantage of wheat/soybean relay intercropping systems. *Scientific Reports*, *13*(1), 1–15. https://doi.org/10.1038/s41598-023-43288-3

Ruillé M, Beillouin D. 2026. Ecological drivers of intercropping performance for enhanced global crop production. *Npj Sustainable Agroculture*, *4*(8), 1–9. https://doi.org/https://doi.org/10.1038/s44264-025-00110-z Ecological

Saraswati E, Gunawati ES, Pudyantini A. 2006. Identifikasi komoditas unggulan sektor pertanian tanaman pangan di wilayah Kabupaten Banyumas. *Eko-Regional*, *1*(2), 117–123.

Simanulang ZA. 2008. *75-Padi-Gogo-Varietas-Situ-Bagendit.*

Singh CM, Singh P, Tiwari C, Purwar S, Kumar M, Pratap A, Singh S, Chugh V, Mishra AK. 2021. Improving drought tolerance in mungbean (*Vigna radiata* L . Wilczek): Morpho-physiological, biochemical and molecular perspectives. *Agronomy*, *11*(1534), 1–20. https://doi.org/https://doi.org/10.3390/agronomy11081534

Sution, Nurdin M. 2015. Keragaan beberapa varietas unggul baru padi sawah irigasi semi teknis tanam pada musim kemarau. *Balai Pengkajian Teknologi Pertanian Kalimantan Barat*, *6*(45), 250–255.

Thilakarathna MS, McElroy MS, Chapagain T, Papadopoulos YA, Raizada MN. 2016. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*, *36*(4), 1–16. https://doi.org/10.1007/s13593-016-0396-4

Turnage G. 2022. Sampling submersed aquatic plant biomass: fresh vs. dry weight. In *Geosystems Research Institute, Mississippi State University*.

Wang W, Wang BZ, Zhang W, Li MY, Li JM, Ji SJ, Abrar M, Rehman MMU, Khan W, Tao HY, Sheteiwy MS, Wang WY, Xiong YC. 2026. Cereal-legume intercropping stabilizes yield and economic advantages under variable rainfall in semiarid rainfed environment. *European Journal of Agronomy*, *174*, 127942. https://doi.org/https://doi.org/10.1016/j.eja.2025.127942

Wang X, Wu Z, Zhou Q, Wang X, Song S, Dong S. 2022. Physiological response of soybean plants to water deficit. *Frontiers in Plant Science*, *12*(809692), 1–12. https://doi.org/10.3389/fpls.2021.809692

Wang X, Zou D, Li C, Zhou W, Li K, Tang Q, Zhu X, Li X, Cao L. 2024. Analysis of characteristics of rice tillering dynamics influenced by sowing dates based on DTM. *Heliyon*, *10*(19).

Wang Y, Han X, Zhao X, Zhang Y. 2024. Grain yield and interspecific competition in an oat-common vetch intercropping system at varying sowing density. *Plant Science*, *15*, 1–13. https://doi.org/10.3389/fpls.2024.1344110

Wang Z, Li Y, Su X, Tao S, Feng X, Wang Q, Xu X, Liu Y, Michaletz ST, Shrestha N, Larjavaara M, Enquist BJ. 2019. Patterns and ecological determinants of woody plant height in eastern Eurasia and its relation to primary productivity. *Journal of Plant Ecology*, *12*(5), 791–803. https://doi.org/10.1093/jpe/rtz025

Wenham K, Williams A, Rossignol T, Collins M. 2019. *Critical period of moisture vulnerability in mungbeans*. GRDC. https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2020/08/critical-period-of-moisture-vulnerability-in-mungbeans

Willey RW, Rao MR. 1980. A competitive ratio for quantifying competition between intercropS. *Expl Agric*, *16*(104), 117–125. https://doi.org/https://doi.org/10.1017/S0014479700010802

Winazira A, Ilyas I, Sufardi S. 2021. Status dan kendala kesuburan tanah pada lahan tegalan dan kebun campuran di Kecamatan Blang Bintang Kabupaten Aceh Besar. *Jurnal Ilmiah Mahasiswa Pertanian*, *6*(2), 79–87. https://doi.org/10.17969/jimfp.v6i2.16950

Wu L, Weston LA, Zhu S, Zhou X. 2023. Rhizosphere interactions: root exudates and the rhizosphere microbiome. In *Frontiers in plant science*, 14, 1–4. https://doi.org/10.3389/fpls.2023.1281010

Xi Y, Du YL, Wang D, Ren JY, Luo WY, Peng Q, Fang WY, Li FM. 2024. Wheat genetic progress in biomass allocation and yield components: A global perspective. *Field Crops Research*, *318*, 109617. https://doi.org/https://doi.org/10.1016/j.fcr.2024.109617

Xu Y, Chu C, Yao S. 2021. The impact of high-temperature stress on rice: Challenges and solutions. *Crop Journal*, *9*(5), 963–976. https://doi.org/10.1016/j.cj.2021.02.011

Yue B, Jin Y, Wu S, Tan J, Chen Y, Zhong H, Chen G, Deng Y. 2025. Research on SPAD inversion of rice leaves at a field scale based on machine vision and leaf segmentation techniques. *Agriculture (Switzerland)*, *15*(12), 1–24. https://doi.org/10.3390/agriculture15121270

Yue X, Hu Y, Zhang H, Schmidhalter U. 2020. Evaluation of both spad reading and spad index on estimating the plant nitrogen status of winter wheat. *International Journal of Plant Production*, *14*(1), 67–75. https://doi.org/10.1007/s42106-019-00068-2

Zhang Y, Liang K, Zhu F, Zhong X, Lu Z, Chen Y, Pan J, Lu C, Huang J, Ye Q, Yin Y, Peng Y, Mo Z, Fu Y. 2024. Differential study on estimation models for indica rice leaf spad value and nitrogen concentration based on hyperspectral monitoring. *Remote Sensing*, *16*(23), 1–16. https://doi.org/10.3390/rs16234604

Zhen X, Huo W, Sanz-Saez A, Miao Y, Chen CY, Batchelor WD. 2024. Drought-tolerant peanut (*Arachis hypogaea* L.) varieties can mitigate negative impacts of climate change on yield in the Southeastern U.S. *Computers and Electronics in Agriculture*, *224*(109105). https://doi.org/https://doi.org/10.1016/j.compag.2024.109105

Zustovi R, Landschoot S, Dewitte K, Verlinden G, Dubey R, Maenhout S, Haesaert G. 2024. Intercropping indices evaluation on grain legume-small grain cereals mixture : a critical meta-analysis review. *Agronomy for Sustainable Development*, *44*(5), 1–21. https://doi.org/10.1007/s13593-023-00934-4

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Laila A · Sulistyorini E

Growth and yield response of shallots to NPK, NK, and humic acid under different mycorrhiza application

Abstract. One strategy to increase shallot production is the utilization of microorganisms in the soil, such as arbuscular mycorrhizal fungi (AMF), which is applied together with fertilizer. This research aimed to investigate the effects of different nutrient inputs and mycorrhizal association on the growth and yield of shallots. This study used a split-plot design with three replications as blocks. The main plot included four treatments: no nutrient inputs, NPK fertilizer, NK fertilizer, and humic acid, while the subplot involved the mycorrhiza application (with or without). The measured variables included growth (plant height and number of leaves) and yield (fresh weight and number of bulbs per plant). The results showed no interaction between treatments for any growth or yield parameters. Application of humic acid significantly improved shallot development and yield, producing the highest results compared to other fertilizer types. Mycorrhizal inoculation enhanced plant height and boosted bulb weight per plant. The application of humic substances has been proven to increase 27.82%, while AMF enhanced 12.9 % weight bulb of shallots. Hence, humic substances have the potential to enhance the shallot with or without AMF application.

Keywords: Arbuscular mycorrhizal fungi · Mycorrhizal inoculation · Humic substances · Shallot bulb yield

Submitted: 15 November 2025, Accepted: 29 April 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.68117

Laila A*[1] · Sulistyorini E[1]
[1] Department of Agroecotechnology, Faculty of Agriculture, University of Sultan Ageng Tirtayasa. Jl. Raya Palka Sindangsari, Pabuaran, Serang 42163, Banten, Indonesia
*Correspondence: alfulaila@untirta.ac.id

Laila A, Sulistyorini E. 2026. Growth and yield response of shallots to NPK, NK, and humic acid under different mycorrhiza application. Jurnal Kultivasi, 25 (1): 28-34.

Introduction

Shallot is one of the horticulture products, used daily as a spice in cuisine or as a traditional medicine. Shallot bulbs contain protein, carbohydrates, fat, minerals, vitamins, and flavonoids (Moreno-rojas et al., 2018). In 2022, the cultivation of shallots in Indonesia covered an area of 122,126 hectares, resulting in a total national production of 1,229,184 tons, which translates to a national productivity of 10.05 tons per hectare. However, shallot productivity has been fluctuating for the last 10 years (BPS, 2022). Meanwhile, the annual demand for shallots is expected to rise to meet the increased production demand. Therefore, it is necessary to improve production to meet the consumers' needs.

One reason for constraints in shallot production is that large farmers use vegetative propagation methods using bulbs. This method has several weaknesses, including the risk of spreading pests and diseases through the bulbs. Vegetative propagation via bulbs causes this issue, as there is no cycle to eliminate pests and pathogens.

Farmers often use synthetic fertilizers such as urea, SP-36, and KCl as nitrogen (N), phosphorus (P), and potassium (K) sources for plants to achieve maximum results. Using inappropriate synthetic fertilizers can affect nutrient absorption by plants, which can lower harvest yields (Liu et al., 2021). In addition, excessive use of NPK can lead to environmental pollution, endanger health, disrupt ecosystem balance, and harm community-supporting biotic production plants (Savci, 2012). One way to improve shallot production is through the utilization of biological fertilizers. Biological fertilizers consist of materials derived from microorganisms that help increase nutrient availability or stimulate plant growth, including vegetables (Adler et al., 2022). Furthermore, the application of biological fertilizers can also reduce pest and pathogen attacks through various mechanisms, such as enhancing microbial activity in the soil, the presence of microbial metabolites, enzyme activity in the soil, and stimulating plant hormone production (Dinesh et al., 2013). A range of microorganisms used as biological fertilizers can help meet essential nutritional needs such as N, P and K.

One of the microorganisms that plays a role as a solvent for phosphorus available to plants is arbuscular mycorrhizal fungi (AMF). Application of AMF in shallots has been proven to increase growth and nutrient absorption efficiency. Research by (Laila et al., 2019) reported that AMF application increased the growth and yield of shallots. Meanwhile, previous study by Warman et al., (2022) showed that the combination of mycorrhiza and organic fertilizer improves crop yield.

One factor influencing colonization by mycorrhiza is the type of nutrient input. Using inorganic fertilizers at appropriate rates can enhance the potential for establishing a symbiotic relationship, whereas organic fertilizers that release nutrients naturally and gradually can support the sustainability of this symbiosis. Mycorrhiza in plants given organic materials, such as humic acid, are believed to improve soil quality, increase soil nutrient content, such as phosphorus, organic materials, and boost crop production. The combination of AMF and humic acid fertilization has been shown to increase growth and tolerance in some crops, but there are few reports on shallots. So, the specific interaction between soil nutrient input types (e.g., NPK, NK, and humic acid) and AMF inoculation in shallots needs to be studied. Therefore, exploring various combinations of AMF with different soil amendments is crucial to identify the optimal synergy for increasing plant productivity while reducing dependence on chemical fertilizers in shallot cultivation.

Materials and Methods

Time and Place Experiment. The study was conducted in Gedung Baya Village, Kalitimbang Subdistrict, District Cibeber, Cilegon City, from May to July 2024, at an altitude of 553 m above sea level. The chemical properties of the soil used are shown in Table 1. The average temperature during May to July fluctuated between 26.1 °C and 29.4°C, while the average humidity ranged from 75% to 97%.

Table 1. Characteristics of soil media used for research

Component	
Chemical Properties	
pH (H_2O)	6.5
KCl	5.7
C-organic (%)	1.08
N Total (%)	0.10
C/N Ratio	11
P_2O_5 (mg /kg)	225.64
K_2O available (mg/kg)	3.08

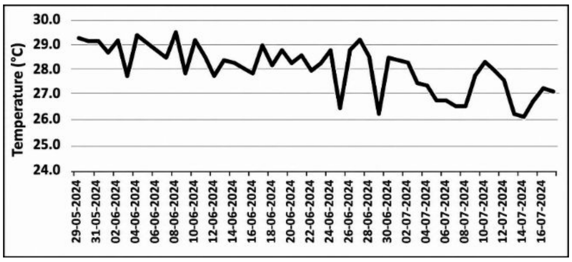


Figure 1. Graph of average temperature (°C) during the study period from May to July 2024

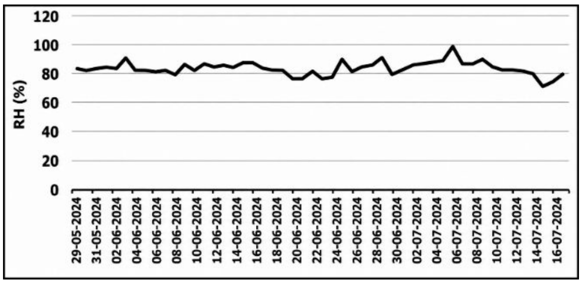


Figure 2. Graph of average relative humidity (%) during the study period from May to July 2024

Materials. Tools used included a hoe, trowel, bucket, plastic, labels, a hand sprayer, and analytical scales. Materials required are seed bulbs of the Bima Brebes variety, soil, urea, TSP, KCl, humic acid, fungicides, and herbicides.

Design Experiment. The study laid out a split-plot design with three blocks as replications. The main plot involved four nutrient input treatments: no nutrient inputs (P0), NPK (P1), NK (P2), and humic acid (P3). The sub-plot included applications of mycorrhiza (M1) and no mycorrhiza application (M0). A total of 24 experimental units were obtained from the treatments. Each experiment plot consisted of 16 plants with 3 sample plants for observation.

Procedure. The land designated for planting has been previously cleared of weeds and other plant remains. Then, raised beds were made with a depth of 30 cm. These raised beds are constructed as high beds, 30 cm in height; the surface beds were loosened and leveled. Planting was done in main plots measuring (2 × 1 m), while the subplots measure (1 × 1 m), with a spacing of 20 cm between plots and blocks. Shallots were planted with a spacing of (20 × 20 cm). Watering was performed daily until the field reached capacity. Fertilizer application followed the specified treatments. In the NPK treatment, fertilizer was applied as a single dose at an N:P:K ratio of 300:100:300 kg.ha^{-1}, using urea, SP-36, and KCl. Urea fertilizer was applied three times, at 2 weeks after planting (WAP), 3 WAP, and 5 WAP. SP-36 and KCl were applied at 2 WAP. The NK treatment involved a combined N and K rate of 300:300 kg.ha^{-1}, using urea and KCl. Urea fertilizer was applied at 2, 3, and 5 WAP, while KCl was applied at 2 WAP. Humic acid fertilizer was applied in powder form at a dose of 200 kg·ha^{-1} two weeks before planting, without adding NPK fertilizer. The absence of fertilizer application means that no treatments were applied. The mycorrhiza treatment was applied one week prior to planting, mixing with soil, using the brand "MycoGrow", at a dose of 10 g per plant. MycoGrow contains 33 spores/g, 300 active propagules/g, and five species of endomycorrhiza on a zeolite medium.

Parameters Observation. Growth parameters included plant height (cm) and the number of leaves (strands) for 3 plant samples per unit, which were measured weekly. The yield parameters consisted of the weight of bulbs per plant (g/plant) and the number of bulbs per plant, at harvest or 56 days after planting.

Data analysis. All observations were analyzed using analysis of variance (ANOVA) for a split-plot design at a 95% confidence level (α = 0.05). The model included main plot effects, subplot effects, and their interaction. When significant differences were detected, mean separation was performed using Duncan's Multiple Range Test (DMRT) at α = 0.05.

Results and Discussion

This research was conducted from May to June 2024, with temperatures ranging from 26.2 to 29.5 ºC (Fig. 1) and relative humidity ranging from 79 to 100% (Fig. 2), indicating that the temperature and humidity are suitable for shallot growing. The soil traits showed that N content was low, P content was high, K was low, organic matter was ideal or moderate, and C/N was optimal (Table 1). It was also indicated that the soil did not require P input, as it was sufficient.

Research results showed that there was no interaction between both factors for all growth and yield parameters. It indicated that soil amendments have different effects on plant height at 1, 2, and 4 WAP. Humic acid could enhance plant height during the initial vegetative stage, reaching 8.25 cm at 1 WAP and 22.92 cm at 2 WAP. The increase continued at the 4 WAP with the plant height reaching 33.92 cm (Table 2). This occurs because the use of humic acid can stimulate the rapid increase in plant height. In plants that received no fertilizer, height was shorter due to delayed sprout emergence, which slows down growth compared to other treatments. However, in the final observation, there is no significant difference.

Humic acid is a naturally occurring material used to repair land, which is produced through the humus extraction process with a high dose (Allahkarami et al., 2025). As an organic fertilizer, humic acid can enhance the physical, chemical, and biological properties of soil (Ampong et al., 2022), including increasing cation exchange capacity (CEC), which enables the land to retain nutrients more effectively, making the soil looser and reducing acidity levels. This occurs because humic acid can improve soil properties by enhancing its chemical and physical state, binding aluminum, which causes acidity, and forming compounds that are difficult to decompose. The content of humic acid includes Sulphur (S), P, oxides of aluminum and iron, sodium (Na), potassium sulfate, magnesium, and a small amount of manganese. Previous studies showed that humic acid improved vegetative growth, including plant height (Rasouli et al., 2022).

Mycorrhiza application had no significant effect on early plant height growth, but it did affect the final observed height. The plant treated with mycorrhiza reached a height of 35.78 cm in the 6 WAP (Table 2). Other research also confirms that applying mycorrhiza can promote plant height growth. AMF absorbs NO_3^- and NH_4^+ through external hyphae, converting them into arginine outside the hyphae, then transferring it to internal hyphae. In the roots, arginine is broken down into ammonium, which plants directly assimilate, thereby increasing nitrogen availability (Bücking and Kafle, 2015). Nitrogen, an essential macronutrient, is readily soluble and easily available to plants, allowing direct root absorption. This element is crucial for vegetative growth, especially for increasing plant height and leaf quantity.

Applying various fertilizers affects shallot leaf growth between 2 and 5 WAP. The highest number of leaves was shown at 2 to 5 WAP when humic acid was applied. The observation results show that the number of leaves was 9.50 at 2 WAP, increasing to 24.33 at 5 WAP. However, by the end of the observation, different soil amendments did not produce varying results in leaf number. The leaf count ranges from 21.44 to 25.06 leaves per plant (Table 3). Humic acid is known to enhance nitrogen absorption, thereby potentially increasing the number of leaves (Ma et al., 2024). This is like other studies that reported humic acid enhanced the number of leaves on crops such as gladiolus (Ahmad et al., 2013) and beans (Qian et al., 2015).

Application of mycorrhiza did not affect the growth or the number of leaves observed over time. The number of leaves in shallots under various mycorrhiza treatments at the end of observation ranged from 24.00 to 24.17 strands (Table 3). Mycorrhizae increased plant height because the external hyphae expand water and nutrient absorption, causing the stem and tissues to elongate more quickly. However, leaf formation is more influenced by genetic factors, so mycorrhizae do not always increase the number of leaves, even when plant height increases.

Table 2. Shallot plant height (cm) in various treatments

Treatment	Week After Planting (WAP)					
	1	2	3	4	5	6
Types of Fertilizer (F)						
Without Fertilizer	6.00 b	20.67 b	28.97 a	31.97 b	33.61 a	35.08 a
NPK	6.83 ab	21.02 ab	29.06 a	32.14 ab	33.00 a	34.69 a
NK	6.67 ab	20.72 b	28.97 a	33.00 ab	33.69 a	34.77 a
Humic acid	8.25 a	22.92 a	30.44 a	33.92 a	34.47 a	35.27 a
AMF (M)						
0 g/plant	6.37 m	20.65 m	28.96 m	32.44 m	33.35 m	34.13 n
10 g/plant	7.50 m	22.01 m	29.76 m	33.06 m	34.04 m	35.78 m
Interaction (F×M)	ns	ns	ns	ns	ns	Ns

Notes: ns = non-significant; Numbers within the same columnn followed by different letters indicate significant differences according to Duncan Multiple Range Test at $p \leq 0.05$.

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

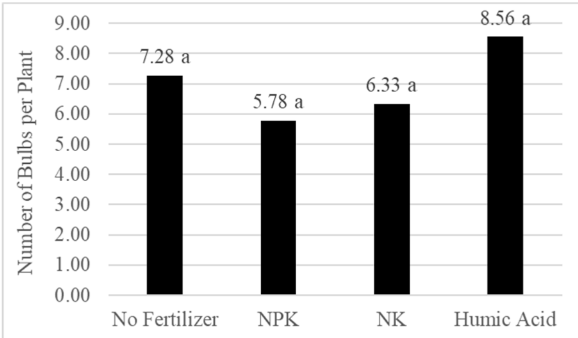
Table 3. Number of leaves (blades) of shallot on various treatments

Treatment	Weeks After Planting (WAP)					
	1	2	3	4	5	6
Types of Fertilizer (F)						
Without Fertilizer	5.27 a	8.39 b	15.44 ab	19.11 bc	21.94 ab	23.89 a
NPK	4.83 a	7.56 b	14.44 b	16.94 c	20.39 b	21.44 a
NK	5.45 a	8.22 b	17.94 ab	21.94 ab	24.61 ab	25.94 a
Humic Acid	7.17 a	9.50 a	19.50 a	22.17 a	24.33 a	25.06 a
AMF (M)						
0 g/plant	5.69 m	8.42 m	16.94 m	20.39 m	22.92 m	24.17 m
10 g/plant	5.67 m	8.42 m	16.72 m	19.69 m	22.72 m	24.00 m
Interaction (F×M)	ns	ns	ns	ns	ns	ns

Notes: ns = non-significant; Numbers within the same columnn followed by different letters indicate significant differences according to Duncan Multiple Range Test at $p \leq 0.05$.

This research showed that variation in fertilizers and humic acid did not affect the number of bulbs per plant, with an average of 5.78 to 8.56 bulbs per plant. Still, applying mycorrhizae produced results that were not significantly different from those without application, with the number of bulbs ranging from 6.81 to 7.17 per plant (Figure 3). Humic acid, one of the kinds of soil amendments that contribute to increasing soil health through improving soil properties and microbial activities (Li et al., 2019), while mycorrhiza application can increase the availability of the element P (Thioub et al., 2019). Phosphorus nutrients in mycorrhizal fertilizers play a crucial role as a structural component in DNA molecules, in energy transfer through ATP, and in regulating phosphate chemistry synthesis, all of which influence plant growth and development (Sbrana et al., 2022). This result indicated that the P soil content is sufficient for the number of shallot bulbs formed. It may indicate that biofertilizer application did not increase some of crop growth and yield parameters because soil nutrients were already available.

Harvested shallot yields varied across different fertilizers and humic acid treatments. Applying humic acid could increase the weight of bulbs per plant from 21.11 g/plant, compared to without fertilization, to 31.59 g/plant (Fig. 4). Humic acid also provides nutrients plants need, although in small amounts. This improvement may be due to humic acid's role in improving the availability of essential macro and micronutrients for photosynthesis and biomass accumulation. Humic substances can also stimulate microbial activity, therefore enhancing nutrient uptake efficiency. These results are similar some research that the crops yield increased after humic acid application (Li et al., 2019).



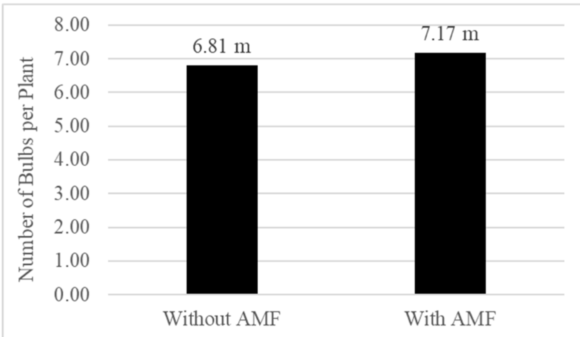
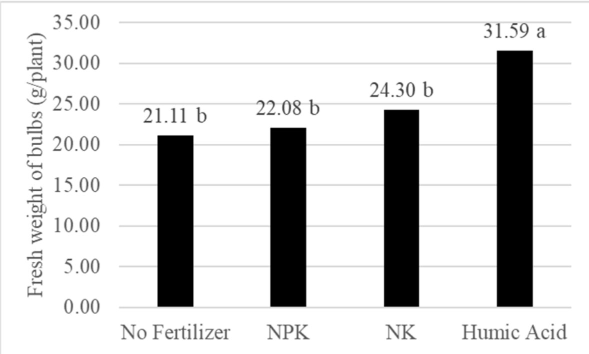


Figure 3. Number of bulbs of shallots on various treatments

Notes: ns = non-significant; Numbers within the same columnn followed by different letters indicate significant differences according to Duncan Multiple Range Test at $p \leq 0.05$**.**



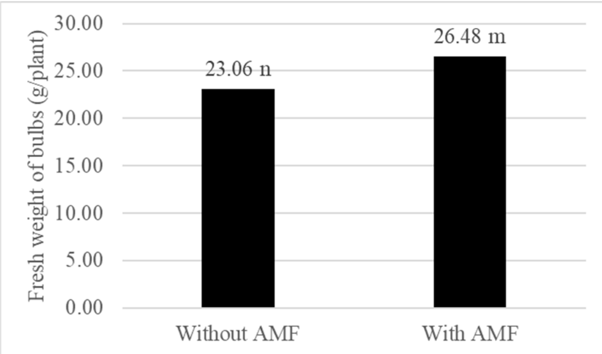


Figure 4. Weights of bulbs of shallot in various treatments

Notes: ns = non-significant; Numbers within the same columnn followed by different letters indicate significant differences according to Duncan Multiple Range Test at $p \leq 0.05$.

Mycorrhiza application could significantly increase shallot bulbs' weight. The shallot that was given mycorrhiza application increased from 23.06 g/plant to 26.48 g/plant (Fig. 4). With proper dosage, mycorrhiza can enhance soil fertility, improve soil properties, and help maintain environmental balance. Other roles of mycorrhiza for plants include facilitating absorption, assimilation, and translocation of nutrients from outside the rhizosphere zone to the plant roots through mycelium (Fall et al., 2022). Based on previous studies, it is well known that mycorrhizae can increase crop yield (Yadav et al., 2023).

Conclusion

Humic acid has the greatest influence on initial vegetative growth and bulb weight, while mycorrhizae increased the maximum plant height and bulb weight. However, there is no significant interaction between the main plot and the subplot on any of the observed parameters. Hence, the use of humic acid and mycorrhizae could become a sustainable strategy to improve shallot productivity while reducing dependence on inorganic fertilizers.

Acknowledgments

This research was funded by LPPM UNTIRTA 2024.

References

Adler C, Amoakwah E, Danquah A, Akaba S, Agyei K, Abonyi T. 2022. Acta Ecologica Sinica Improved soil physicochemical , biological properties and net income following the application of inorganic NPK fertilizer and biochar for maize production. Acta Ecologica Sinica, 42(4), 289–295. https://doi.org/10.1016/j.chnaes.2021.12.002

Ahmad I, Saquib RU, Qasim M, Saleem M, Khan AS, Yaseen M. 2013. Humic acid and cultivar effects on growth, yield, vase life, and corm characteristics of gladiolus. Chilean Journal of Agricultural Research, 73(4), 339–344. https://doi.org/10.4067/S0718-58392013000400002

Allahkarami E, Allahkarami E, Rezai B, Azadmehr A. 2025. Advancing humic acids extraction procedures : A review of methods , innovations , and sustainability challenges. Separation and Purification Technology, 376(P1), 133954. https://doi.org/10.1016/j.seppur.2025.133954

Ampong K, Thilakaranthna MS, Gorim LY. 2022. Understanding the Role of Humic Acids on Crop Performance and Soil Health. 4(March). https://doi.org/10.3389/fagro.2022.848621

Dinesh R, Anandaraj M, Kumar A, Srinivasan V, Bini YK, Subila KP, Aravind R, Hamza S. 2013. Effects of Plant Growth-Promoting Rhizobacteria and NPK Fertilizers on Biochemical and Microbial Properties of Soils Under Ginger (Zingiber officinale) Cultivation. 2(December), 346–353. https://doi.org/10.1007/s40003-013-0080-8

Fall AF, Nakabonge G, Ssekandi J. 2022. Roles of Arbuscular Mycorrhizal Fungi on Soil Fertility : Contribution in the Improvement of Physical , Chemical , and Biological

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Properties of the Soil. 3(March), 1–11. https://doi.org/10.3389/ffunb.2022.723892

Laila A, Trisnaningrum N, Hamawi M. 2019. Significant potential of arbuscular mycorrhizae fungi to increase on yield of shallot. IOP Conference Series: Earth and Environmental Science, 292(1). https://doi.org/10.1088/1755-1315/292/1/012017

Li Y, Fang F, Wei J, Wu X, Cui R, Li G, Zheng F, Tan D. 2019. Humic Acid Fertilizer Improved Soil Properties and Soil Microbial Diversity of Continuous Cropping Peanut: A Three-Year Experiment. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-48620-4

Liu B, Wang X, Ma L, Chadwick D, Chen X. 2021. Combined applications of organic and synthetic nitrogen fertilizers for improving crop yield and reducing reactive nitrogen losses from China ' s vegetable systems : A meta-analysis *. Environmental Pollution, 269, 116143. https://doi.org/10.1016/j.envpol.2020.116143

Ma Y, Cheng X, Zhang Y. 2024. The Impact of Humic Acid Fertilizers on Crop Yield and Nitrogen Use Efficiency : A Meta-Analysis. 1–12.

Moreno-rojas R, Pérez-aparicio J, Pereira-caro G. 2018. LWT - Food Science and Technology Development and validation of UHPLC-HRMS methodology for the determination of fl avonoids, amino acids and organosulfur compounds in black onion , a novel derived product from fresh shallot onions (Allium cepa var . aggregatum). LWT - Food Science and Technology, 97(May), 376–383. https://doi.org/10.1016/j.lwt.2018.07.032

Qian S, Ding W, Li Y, Liu G, Sun J, Ding Q. 2015. Characterization of humic acids derived from Leonardite using a solid-state NMR spectroscopy and effects of humic acids on growth and nutrient uptake of snap bean. Chemical Speciation and Bioavailability, 27(4), 156–161. https://doi.org/10.1080/09542299.2015.1118361

Rasouli F, Nasiri Y, Asadi M, Hassanpouraghdam MB, Golestaneh S, Pirsarandib Y. 2022. Fertilizer type and humic acid improve the growth responses, nutrient uptake, and essential oil content on Coriandrum sativum L. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-11555-4

Savci S. 2012. Investigation of Effect of Chemical Fertilizers on Environment. 1(January), 287–292. https://doi.org/10.1016/j.apcbee.2012.03.047

Sbrana C, Agnolucci M, Avio L, Giovannini L, Palla M. 2022. Mycorrhizal Symbionts and Associated Bacteria : Potent Allies to Improve Plant Phosphorus Availability and Food Security. 12(January), 1–6. https://doi.org/10.3389/fmicb.2021.797381

Thioub M, Ewusi-mensah N, Sarkodie-addo J, Adjei-gyapong T. 2019. Soil & Tillage Research Arbuscular mycorrhizal fungi inoculation enhances phosphorus use efficiency and soybean productivity on a Haplic Acrisol. Soil & Tillage Research, 192(October 2018), 174–186. https://doi.org/10.1016/j.still.2019.05.001

Warman R, Santari PT, Sandi N. 2022. Performance of Shallots (Allium Ascalonicum L) in Peat Soil with Organic Fertilizer and Arbuscular Mycorrhizal Fungi (AMF). Jurnal Penelitian Pendidikan IPA, 8(SpecialIssue), 58–66. https://doi.org/10.29303/jppipa.v8ispecialissue.2482

Yadav VK, Kumar D, Jha RK, Bairwa RK, Singh R, Mishra G, Singh JP, Kumar A, Vinesh B, Jayaswall K, Rai AK, Singh AN, Kumar S, Rajavat MVS, Jayaswal D. 2023. Mycorrhizae set the stage for plants to produce a higher production of biomolecules and stress-related metabolites: a sustainable alternative of agrochemicals to enhance the quality and yield of beetroot (Beta vulgaris L.). Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1196101

ISSN: 1412-4718, eISSN: 2581-138x

Marpaung AE · Sabrina T · Rauf A · Susilowati DN

Effects of phosphate-solubilizing fungi on phosphorus availability, retention, and soil chemical properties in shallot cultivated on andisols of North Sumatra

Abstract. The availability of phosphorus (P) is one of the main limiting factors for plant productivity in Andisol soils due to the strong fixation of P by allophane clay minerals, aluminum and iron oxides. Giving the phosphate solubilizing fungi (PSF) is an alternative to increase available P which plants can use to increase the yield. This study aimed to evaluate the effectiveness of phosphate-solubilizing fungi (PSF) in improving P availability on Andisol. The experiment used a block randomized design (BRD) with three replications. The first factor is Andisol soil origin (Soil 1 and Soil 2) and the second is isolate of phosphate solubilizing fungi (*Aspergillus niger* P13, *A. niger* P21, *A. pseudodeflectus* BJ21, and *A. niger* BJ23). The research results showed that Soil 1 generally produces higher growth and yield of shallots than soil 2. *A. pseudodeflectus* BJ21 can increase available P by 48.46% and *A. niger* P13 by 45.71% compared to without phosphate solubilizing fungi. *A. niger* P13 can reduce P retention by 18.48% compared to without phosphate solubilizing fungi. *A. niger* BJ23 can increase plant P uptake by 35.35% compared to without phosphate solubilizing fungi. Inoculation of isolates *A. niger* P13 and *A. pseudodeflectus* BJ21 was able to adapt and grow well on Andisol soil which significantly increased the population compared to the control. Indigenous phosphate-solubilizing fungi (*A. niger* and *A. pseudodeflectus*) improved P availability and uptake in Andisol, reduced P retention, adapted well to the soil environment, and showed strong potential as environmentally friendly biofertilizers based on local resources.

Keywords: *Allium cepa* L · *Aspergillus niger* · *Aspergillus pseudodeflectus* · Phosphat · Yield.

Submitted: 19 November 2025, Accepted: 31 March 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.68191

Marpaung AE[1,3] · Sabrina T[2*] · Rauf A[2] · Susilowati DN[3]
[1] Graduate School of Agriculture Science, Universitas Sumatera Utara, Jl. Dr. A. Sofian No.3, Padang Bulan, Medan Baru, Medan 20155, North Sumatra, Indonesia
[2] Faculty of Agriculture, Universitas Sumatera Utara, Jl. Dr. A. Sofian No.3, Padang Bulan, Medan Baru, Medan 20155, North Sumatra, Indonesia
[3] Research Center for Horticultural, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta - Bogor KM 46, Cibinong 16911, West Java, Indonesia
*Correspondence: t.sabrina@usu.ac.id

Marpaung AE, Sabrina T, Rauf A, Susilowati DN. 2026. Effects of phosphate-solubilizing fungi on phosphorus availability, retention, and soil chemical properties in shallot cultivated on andisols of North Sumatra. Jurnal Kultivasi, 25 (1): 35-43.

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Andisol (Andosol) is a volcanic ash–derived soil characterized by the dominance of short-range-order (amorphous) minerals such as allophane, imogolite, and ferrihydrite as well as Al–humus complexes, which confer unique physical and chemical properties including low bulk density and high porosity, high water-holding capacity, and a dark, friable surface horizon rich in organic matter with relatively high cation exchange capacity. These colloidal constituents also influence organic carbon stabilization through organo-mineral complexation, which is generally fertile but phosphorus-challenged (Takahashi & Dahlgren 2016).

Phosphorus (P), an essential nutrient, is responsible for photosynthesis, root formation, flowering, and bulb formation in shallots. Crop production is often affected by phosphate deficiency in many agricultural systems, particularly in Andisol soils, which are formed from volcanic ash and rich in allophane minerals and Al/Fe oxides, leading to high phosphate retention and reduced phosphate availability to plants (Marpaung et al., 2024a).

Most of the P nutrients found in soil are insoluble. Soil contains only around 0.1% of all plant P (Zhu et al., 2018). (Mehta et al., 2013), states that the biogeochemical cycle's fixation reaction mechanism results in cation deposition in the soil, immobilization, adsorption, and interconversion to organic forms. Large soil P reserves exist, but they are not readily available (Kishore et al., 2015). In order to get the best possible crop output, this leads farmers to apply a lot of inorganic phosphate fertilizer (Sharma et al., 2015). Low phosphate (P) availability is a concern in almost all tropical soils, including Andisol. High phosphate retention is caused by Andisol 97.8% concentration of allophane, the most reactive clay mineral (Sukarman & Dariah, 2014). Phosphate adsorption thus reduces the amount of accessible phosphate (P) in the soil, making phosphate unavailable to plants (Sukarman & Dariah, 2014; Marpaung et al., 2021).

High doses of inorganic phosphate fertilizers are traditionally used to manage phosphorus in Andisols. However, this method is not always effective because most of the applied phosphorus is bound and becomes unavailable. Alternative strategies that increase the utilization of phosphorus stored in the soil and reduce the need for inorganic fertilizers are needed because, in addition to efficiency issues, reliance on inorganic phosphorus fertilizers also poses economic challenges for farmers and has long-term negative environmental impacts (Silva et al., 2023).

The use of phosphate-solubilizing microorganisms (including phosphate-solubilizing bacteria and fungi) is one approach that has received attention. Phosphate-solubilizing fungi (PSF) have the ability to solubilize limited inorganic phosphate through enzyme secretion, organic acid production, and release. Several laboratory and field studies have shown that PSF inoculation can increase soil-available phosphorus, plant phosphorus uptake, growth parameters, and yield in various food crops. However, the performance of PSF is strongly influenced by soil type, fungal species, environmental conditions, and fertilization techniques used (Arias et al., 2023). Research results from Marpaung, et al. (2024b) had been produced on phosphate-solubilizing fungi which can increase plant growth and yield. The phosphate-dissolving fungi *A. niger* PSF-8 isolated from mangrove plants is capable of dissolving phosphate and has the potential to be used as a bio fertilizer (Bhattacharya et al., 2015). Elias, et al. (2016a) found that the application of the phosphate-solubilizing fungi *Aspergillus* sp and *Penicilliun* sp can be able to increase growth, soil P and N content, and yield of *Phaseolus vulgaris* L.

The aim of this study was to evaluate the effect of phosphate-solubilizing fungi inoculation on soil available P, plant P uptake, and shallot yield on Andisol. This study hypothesizes that applying phosphate-solubilizing fungi to several soil sources can increase soil available P, plant P uptake, and shallot yield on Andisol.

Materials and Methods

The experiment was carried out in plastic houses located in Karo Regency, Indonesia, from January to June 2024. A factorial randomized block design was employed in the study. The treatment consisted of two factors: the first factor was the soil origin (soil 1 and soil 2). The second factor is the type of phosphate solubilizing fungi (Control, *A. niger* P13, *A. niger* P21, *A. pseudodeflectus* BJ21, *A. niger* BJ23). Each treatment consisted of 10 plants and was replicated three times.

Two Andisol samples were used: soil 1 collected from Dolat Rayat, Karo Regency, and soil 2 collected from Siborong-borong, North Tapanuli Regency. Isolate of phosphate solubilizing fungi is collection of Agriculture faculty of USU and BRIN from shallot rhizosphere. Isolate multiplication activities were carried out at the USU Laboratory. Phosphate-solubilizing fungal inoculants are prepared by first growing them on PDA media and incubating them for 2-4 days at 28^{0}C. Each pure culture of phosphate-solubilizing fungi was made until the fungal population density reached around 10^8 CFUml^{-1} for the treatment. Each treatment's planting media was a 2:1 mix of soil and chicken manure. After the media has cooled and been sterilized, it is placed in a 3 kg polybag and covered with sterile water until it reaches field capacity. In a screen/plastic house, shallot seeds of the Batu Ijo variety are planted in polybags and treated with chemical fertilizers N 175 kg ha^{-1}, P_2O_5 90 kg ha^{-1}, and K_2O 120 kg ha^{-1} both during and one month after planting. Phosphate-solubilizing microbes were applied one week after planting at a dose of two millilitres per plant and five weeks after planting at a dose of ten millilitres per plant under the tested treatment, which was administered by watering it into the planting hole in the morning (Sriwantoko et al., 2020). Vegetable pesticides are sprayed as part of maintenance to stop pest and disease infestations. Depending on the severity of pest or plant disease attacks, spraying was done every four days. When the plants are 70–80 days following planting, harvesting can start.

The observation variables were secondary parameter is soil chemical properties before treatment and primary parameter are P available, P retention, P absorption (composite soil was taken from each treatment at 7 weeks after planting), pH, C-organic, and phosphate solubilizing fungi population in soil. The mean for each observation variable was analyzed using the F test and continued with the Tukey HSD test at the 5% level.

Results and Discussion

Soil Chemical Properties Before Treatment. The results of the study of the chemical characteristics of the soil before treatment are shown in Table 1. The chemical characteristics of the soil before treatment showed that the soil in Soil 1had high levels of C-organic, N, and K_2O (4.91%, 52%, and 0.64 mg/100 g-1, respectively). The P_2O_5 (available P) content of the soil was relatively low, namely 2.36 ppm, while the total P (HCl 25%) and P retention was relatively high and very high, namely 54.45 mg/100 g^{-1} and 97.80%, respectively. In Soil 2, the C-organic content was high (4.37%), while the N and K_2O content were moderate (0.48% and 0.40 mg/100 g^{-1}, respectively). The P_2O_5 (available P) content of the soil was low, namely 2.21 ppm, while the total P (HCl 25%) and P retention was high and very high, namely 49.31 mg/100 g^{-1} and 93.90%, respectively. Soil pH in soil 1 and soil 2 is slightly acidic.

P Available, P Retention and P Absorption. The available P content of the soil is influenced by the interaction of the two treatments of soil origin and the type of PSF (Table 2). In general, the soil origin of Soil 2 produces significantly higher available P than Soil 1 Regency, namely 239.78 ppm compared to 192.72 ppm, respectively. The application of PSF, produces higher available P than the treatment without PSF, except for the PSF type of *A. niger* BJ23. In general, *A. niger* P13 and *A. niger* P21 produce significantly higher available P than the other treatments, namely 250.19 ppm and 235.26 ppm, respectively.

Table 1. Soil chemical properties before treatment

	Soil 1		Soil 2	
Criteria	**Value**	**Note***	**Value**	**Note***
C-organic (%)	4.91	High	4.37	High
P_2O_5 – Bray 1 (ppm)	2.36	Low	2.21	Low
P_2O_5 HCl 25% (mg/100 g)	54.45	High	49.31	High
P-Retention (%)	97.80	Very high	93.90	Very high
pH H_2O	5.9	Slightly acidic	5.88	Slightly acidic

ISSN: 1412-4718, eISSN: 2581-138x

Table 2. Interaction of soil origin and type of phosphate solubilizing fungi on P available

Treatments	P Available (ppm) Andisol soil origin		Average
	Soil 1	Soil 2	
Without PSF	143.35 d	242.54 ab	**192.95 abc**
A. niger P13	264.06 a	236.32 ab	**250.19 a**
A. niger P21	225.50 abc	245.03 ab	**235.26 a**
A. pseudodeflectus BJ21	165.07 cd	281.53 a	**223.30 ab**
A. niger BJ23	165.73 cd	193.50 bcd	**179.61 c**
Average	**192.74 b**	**239.78 a**	
CV (%)	**11.39**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

Table 3. Interaction of soil origin and type of phosphate solubilizing fungi on P retention

Treatments	P Retention (%) Andisol soil origin		Average
	Soil 1	Soil 2	
Without PSF	98.58 a	87.48 de	**93.03 a**
A. niger P13	80.36 f	88.62 cde	**84.49 c**
A. niger P21	92.24 bc	87.09 e	**89.67 b**
A. pseudodeflectus BJ21	97.48 a	90.11 cde	**93.79 a**
A. niger BJ23	96.16 ab	91.08 cd	**93.62 a**
Average	**92.96 a**	**88.88 b**	
CV (%)	**1.51**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

The interaction of the two treatments was able to increase the available P content of the soil, where the application of *A. pseudodeflectus* BJ21 in the soil origin of Soil 2 resulted in the highest available P, which was 281.53 ppm and was not significantly different from the treatment of *A. niger* P13 in the soil origin of Soil 1 Regency, which was 264.06 ppm. Meanwhile, the lowest available P was found in control treatment in the soil origin of Soil 1 Regency, which was 143.35 ppm. This indicates that the application of PSF was able to dissolve bound P so that the available P increased compared to the soil before treatment, which was 2.21-2.36 ppm.

The interaction between the two treatments of soil origin and PSF type significantly affected the P content of soil retention (Table 3). The soil origin of Soil 1generally produced significantly higher P retention than that of Soil 2, except for the PSF type *A. niger* P13 in the soil origin of Soil 1which produced the lowest P retention.

The interaction of the two treatments significantly affected P retention, with the lowest P retention occurring in soil from soil 1 with *Aspergillus niger* P13 as PSF, at 80.36%. The highest P retention was found in soil from soil 1 without PSF application, at 98.58%. This suggests that PSFF plays an important role in increasing phosphorus (P) solubility for plants by reducing P retention in the soil. The main mechanisms used by PSF in dissolving P include the production of organic acids, phosphatase enzymes, and siderophores.

The application of the PSF type treatment significantly affected plant P uptake, while the soil origin treatment and the interaction between the two treatments did not significantly affect it. The soil from soil 1 resulted in higher P uptake in shallots than the soil from soil 2, although the statistical difference was not significant (Table 4).

P uptake by shallot plants with PSF application on shallot plants resulted in significantly higher P uptake than without PSF application, except for the Aspergillus niger P21 treatment. Application of PSF type *A. niger* BJ23 resulted in the highest P uptake by plants, which was 1.98% and the lowest was found in the control treatment, which was 1.28%. The type of *A. niger* BJ23 produced P uptake that was not significantly different from the treatments of *A. pseudodeflectus* BJ21 and *A. niger* P13. This shows that providing the right type of phosphate-solubilizing microbes will help the availability of P that can be directly absorbed by plants.

Table 4. Effect of soil origin and type of phosphate solubilizing fungi on P absorption

Treatments	P Absorption (mg plant^{-1})
Soil origin	
Soil 1	1.63
Soil 2	1.45
Phosphate solubilizing fungi	
Without PSF	1.28 b
A. niger P13	1.51 ab
A. niger P21	1.28 b
A. pseudodeflectus BJ21	1.65 ab
A. niger BJ23	1.98 a
CV (%)	**19.85**

Note: Means followed by the same letter on the same columns is not significant different by Tukey HSD test at 5% level

Table 5. Effect of soil origin and type of phosphate solubilizing fungi on pH and C-organic

Treatments	pH	C-Organic
Soil origin		
Soil 1	6.10 a	7.59 a
Soil 2	5.82 b	6.07 b
Phosphate solubilizing fungi		
Without PSF	6.10	6.70
A. niger P13	5.95	6.88
A. niger P21	5.95	6.91
A. pseudodeflectus BJ21	5.92	6.92
A. niger BJ23	5.88	6.73
CV (%)	**3.47**	**5.31**

Note: Means followed by the same letter on the same columns is not significant different by Tukey HSD test at 5% level

pH and Organic Carbon Soil. Soil chemical properties showed that soil origin treatment had a significant effect on soil pH and C-organic, while phosphate-solubilizing fungus type treatment and the interaction of the two treatments had no significant effect (Table 5). Soil origin treatment gave a significant difference between treatments on soil pH and C-organic, where soil origin from Soil 1produced significantly higher pH and C-organic than soil origin from Soil 2, namely 6.10 and 7.59%, respectively.

This is consistent with the higher pH and organic carbon content before treatment. After treatment, the pH and organic carbon of each soil source increased. This is related to the addition of phosphate-solubilizing microbes, resulting in an increase in pH and organic carbon. In general, the application of PSF resulted in a lower pH compared to the treatment without phosphate-solubilizing fungi. Meanwhile, organic carbon content increased after the PSF treatment, although not statistically significantly.

Phosphate Solubilizing Fungi Population. The results showed that the type of phosphate solubilizing fungi (PSF) and soil origin (Soil 1 and Soil 2) influenced the population of phosphate solubilizing fungi in Andisol soil. In general, Soil 2 produced a larger PSF population than Soil 1, with an average value of 7.53 × 10^8 CFU g^{-1}. The treatment without PSF (control) showed the lowest PSF population in both soil types, namely 1.87 × 10^8 CFU g^{-1} in Soil 1 and 3.57 × 10^8 CFU g^{-1} in Soil 2. Compared with the control, inoculation of phosphate solubilizing fungi significantly increased the PSF population. The population values of *A. niger* P13 and *A. pseudodeflectus* BJ21 were the highest (8.75 × 10^8 and 7.42 × 10^8 CFU g^{-1}), but *A. niger* BJ23 was the lowest (5.01 × 10^8 CFU g^{-1}). The interaction between soil origin and PSF type showed that some isolates, especially *A. niger* P13, were able to grow better on Soil 2 than Soil 1 (Table 6).

Table 6. Effect of soil origin and type of phosphate solubilizing fungi on phosphate solubilizing fungi population

Treatments	Phosphate Solubilizing Fungi Population (10^8 CFU g^{-1}) Andisol soil origin		Average
	Soil 1	Soil 2	
Without PSF	1.87 c	3.57 bc	**2.72 b**
A. niger P13	6.84 ab	10.66 a	**8.75 a**
A. niger P21	6.25 abc	6.84 ab	**6.55 a**
A. pseudodeflectus BJ21	4.28 bc	10.56 a	**7.42 a**
A. niger BJ23	3.98 bc	6.04 abc	**5.01 b**
Average	**4.64 b**	**7.53 a**	
CV (%)	**1.36**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

The soil chemical composition before treatment showed several characteristics of Andisol, namely high organic carbon content, acidic-slightly acidic pH, low P_2O_5 (available P), and high P retention. This is in accordance with Anindita et al. (2023), which states that Andisols, particularly those under forest or agricultural land use, can contain significant amounts of organic carbon due to interactions with amorphous minerals like allophane that help protect carbon. These characteristics make Andisol suitable for agricultural use. This property is also consistent with the characteristics of Andisol, which includes low available phosphorus due to significant phosphorus fixation by allophane (Zhu et al., 2018). The soil pH value ranged from 5.88 to 5.90, classified as slightly acidic. According to (Sukarman & Dariah, 2015), most Andisol in Indonesia are acidic, with a pH range of 3.4 to 6.7. Soil 1 has higher organic C, N, and K_2O content, thus providing a better source of energy and nutrients for plants. However, Soil 1 also has very high P retention, so that initial P availability remains low even though total P is high. In contrast, Soil 2 has still high organic C but lower N and K_2O, so that soil fertility is relatively more limited, but the slightly lower P retention value compared to Soil 1 allows phosphate-solubilizing fungi to develop better and be more effective in dissolving bound P.

In Andisol soil, the relative surface charge of the cell will bind with the charge of the soil clay minerals, thereby reducing the adsorption capacity of the clay minerals to phosphate (Herzberg & Elimelech, 2008). Additionally, by obstructing or competing for soil adsorption sites on Al and Fe oxides, organic acid anions generated by phosphate-solubilizing microorganisms can decrease phosphate fixation and increase soil phosphorus availability(Alori et al., 2017). Furthermore, P absorption activity through the release of organic acids such as fumarate, lactate, and citrate can break down P ions bound to soil cations such as Al, Ca, Fe, and Mg and then convert them into forms available for plant absorption (Tian et al., 2021; Timofeeva et al., 2022). The principle of the phosphate mineral dissolution mechanism is the production of organic acids and the acid phosphatase enzyme, which plays a role in the mineralization of organic phosphate in the soil (Setiawati & Pranoto, 2015). According to (Istina & Nurhayati, 2019), native phosphate-solubilizing microbes can increase the soil P nutrient content.

Phosphate-solubilizing microbes produce organic acids such as citric, oxalic, and malic acids that can dissolve inorganic phosphate bound to soil minerals, thereby increasing the availability of P for plants. Producing phosphatase enzymes, PSF secrete phosphatase enzymes that hydrolyze organic phosphate compounds into inorganic forms that can be absorbed by plants. Producing siderophores, some PSF produce siderophores that chelate metal ions such as Fe^{3+} and Al^{3+}, which normally bind phosphate in the soil. By binding these metal ions, phosphate becomes more available to plants and P soil retention will decrease. Insoluble phosphate can be dissolved and mineralized using different phosphate-solubilizing microbes that produce phosphatase enzymes, low-molecular-weight organic acids (Zhu et al., 2018). According to Campos, et al. (2018), the activity of the PSF group that is able to secrete organic acids can extract phosphate from an insoluble form to become available so that plants can absorb P elements to meet their needs.

Phosphate-solubilizing microbes have the ability to dissolve bound P into plant-available P through the production of organic acids, phosphatase enzymes, and siderophores. *A. niger* is a phosphate-solubilizing microbe that can dissolve insoluble P compounds (Rojas et al., 2019). These microorganisms can increase phosphorus uptake by releasing phosphorus compounds bound to the soil and making them available to plants (Kumar et al., 2018). (Baumann et al., 2018) stated that phosphate-solubilizing microorganisms can dissolve fixed phosphorus by releasing organic acids, namely by lowering pH, through chelation activity, competing with phosphorus for adsorption sites, and releasing phosphorus through the formation of soluble complexes with metal ions that will bind phosphorus strongly. The excretion of organic acids by phosphate-solubilizing microbes will chelate Fe and Al ions so that P is released. In addition, PSM produces phosphatase enzymes that play a role in the mineralization of organic P so that orthophosphate release occurs from soil Po sources (Bi et al., 2020). Siderophores are also produced by several types of phosphate-solubilizing microbes, which are a group of secondary metabolites with high affinity for Fe (Gu et al., 2020), so that Fe will be chelated and P will be released.

Soil acidity is a crucial factor in ensuring phosphorus immobilization in the soil (Zhu et al., 2018). Microbial growth and activity are significantly influenced by changes in the acidity of their environment. Bacteria generally have maximum solubility at pH 5.5-6, while fungal spores have a pH of 5-6. The pH activity of fungal isolates decreased significantly at pH 7-8. Research by (Wang et al., 2021) indicates that a lower pH does not necessarily translate to a higher P solubilization capacity. However, according to Li, et al. (2019), there is a strong correlation between decreased pH and P solubilization.

The application of PSF increased the population of phosphate-solubilizing fungi in Andisol soil. This indicates that the applied fungi have the ability to thrive and adapt well to volcanic soils (Takahashi & Dahlgren, 2016); (Takahashi & Dahlgren, 2016; Kalayu, 2019). This soil has a high phosphate fixation capacity due to the presence of active Al and Fe, but the unique characteristics of Andisols, which are rich in organic matter and dominated by amorphous minerals such as allophane and imogolite, provide physical conditions that support the growth of microorganisms in the soil (Takahashi & Dahlgren, 2016). Initial soil characteristics, such as organic matter content, pH, and soil chemistry, influence the success of phosphate-solubilizing fungi colonization. Differences in fungal populations in Soil 1 and Soil 2 demonstrate this (Alori et al., 2017). Populations of *A. niger* P13 and *A. pseudodeflectus* BJ21 isolates were larger than those of other isolates; This indicates a better physiological and metabolic ability to utilize soil carbon sources and dissolve bound phosphate through the production of organic acids such as oxalic and citric acids (Kalayu 2019). These results indicate that the compatibility between microbial isolates and Andisol soil characteristics greatly influences the effectiveness of phosphate-solubilizing fungus-based biofertilizers (Alori et al., 2017; Takahashi & Dahlgren, 2016). According to the results of research by Elias et al., 2016b), the *Aspergillus* genus can dissolve phosphate qualitatively.

The results of the study on the use of indigenous phosphate-solubilizing fungal isolates *A. niger* and *A. pseudodeflectus* in Andisol soil were able to improve soil chemical properties by increasing organic carbon, available P, P uptake by shallot plants in particular and reducing P retention. In addition, this type of fungus can also adapt and grow well. This shows the potential for developing agricultural technology based on local resources. The use of phosphate-solubilizing fungi as biofertilizers supports a more efficient and environmentally friendly food production system by reducing dependence on inorganic phosphate fertilizers.

Conclusion

Soil 1 generally produces higher growth and yield of shallots than soil 2. *A. pseudodeflectus* BJ21 can increase available P by 48.46% and *A. niger* P13 by 45.71% compared to without phosphate solubilizing fungi. *A. niger* P13 can reduce P retention by 18.48% compared to without phosphate solubilizing fungi. *A. niger* BJ23 can increase plant P uptake by 35.35% compared to without phosphate solubilizing fungi. Application of phosphate solubilizing fungi can increase shallot yields by 63.19%-72.39% compared to without phosphate solubilizing fungi. Inoculation of isolates *A. niger* P13 and *A. pseudodeflectus* BJ21 was able to adapt and grow well on Andisol soil which significantly increased the population compared to the control.

Acknowledgments

The research was funded by an internal project of the Research Organization for Food and Agriculture 2023 under project number 9/III.11/HK/2023, for which the author is grateful to the Research Organization for Agriculture and Food (BRIN), Indonesia.

References

Alori ET, Glick BR, Babalola OO. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology, 8: 971. https://doi.org/10.3389/fmicb.2017.00971

Anindita S, Finke P, Sleutel S. 2023. Tropical Andosol organic carbon quality and degradability in relation to soil geochemistry as affected by land use. SOIL, 9: 443–459. https://doi.org/10.5194/soil-9-443-2023

Arias RM, Heredia Abarca G, del Carmen Perea Rojas Y, de la Cruz Elizondo Y, García Guzman KY. 2023. Selection and Characterization of Phosphate-Solubilizing Fungi and Their Effects on Coffee Plantations. Plants, 12: 3395. https://doi.org/10.3390/plants12193395

Baumann K, Jung P, Samolov E, Lehnert LW, Büdel B, Karsten U, Bendix J, Achilles S, Schermer M, Matus F, Oses R, Osses P, Morshedizad M, Oehlschläger C, Hu Y, Klysubun W, Leinweber P. 2018. Biological soil crusts along a climatic gradient in Chile: Richness and imprints of phototrophic microorganisms in phosphorus biogeochemical cycling. Soil Biology and Biochemistry, 127: 286–300. https://doi.org/10.1016/J.SOILBIO.2018.09.035

Bhattacharya S, Das A, Bhardwaj S, Rajan SS. 2015. Phosphate solubilizing potential of aspergillus niger PSF-8 isolated from muthupettai mangrove. Journal of Scientific and Industrial Research, 74: 499–503.

Bi QF, Li KJ, Zheng BX, Liu XP, Li HZ, Jin BJ, Ding K, Yang XR, Lin XY, Zhu YG. 2020. Partial replacement of inorganic phosphorus (P) by organic manure reshapes phosphate mobilizing bacterial community and promotes P bioavailability in a paddy soil. Science of the Total Environment, 703. https://doi.org/10.1016/j.scitotenv.2019.134977

Campos P, Borie F, Cornejo P, López-Ráez JA, López-García Á, Seguel A. 2018. Phosphorus acquisition efficiency related to root traits: Is mycorrhizal symbiosis a key factor to wheat and barley cropping? Frontiers Plant Science, 9: 1-21. Frontiers Media S.A. https://doi.org/10.3389/fpls.2018.00752

Elias F, Muleta D, Woyessa D. 2016a. Effects of Phosphate Solubilizing Fungi on Growth and Yield of Haricot Bean (*Phaseolus vulgaris* L.) Plants. Journal of Agricultural Science, 8: 204. https://doi.org/10.5539/jas.v8n10p204

Elias F, Woyessa D, Muleta D. 2016b. Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. International Journal of Microbiology, 2016: 1-11. https://doi.org/10.1155/2016/5472601

Gu S, Wei Z, Shao Z, Friman VP, Cao K, Yang T, Kramer J, Wang X, Li M, Mei X, Xu Y, Shen Q, Kümmerli R, Jousset A. 2020. Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. Nature Microbiology, 5: 1002–1010. https://doi.org/10.1038/s41564-020-0719-8

Herzberg M, Elimelech M. 2008. Physiology and genetic traits of reverse osmosis membrane biofilms: A case study with *Pseudomonas aeruginosa*. ISME Journal, 2: 180–194. https://doi.org/10.1038/ismej.2007.108

Istina IN, Nurhayati J. 2019. Indigenous Phosphate Solubilizing Microbe on Peat Soil Productivity Enhance at Riau Province. International Journal of Science and Research, 8: 1572-1576. https://www.researchgate.net/profile/Nurhayati-Nurhayati-32/publication/380182767

Kalayu G. 2019. Phosphate solubilizing microorganisms: Promising approach as biofertilizers. International Journal of Agronomy, 2019, 4917256. https://doi.org/10.1155/2019/4917256

Kumar MS, Reddy GC, Phogat M, Korav S. 2018. Role of bio-fertilizers towards sustainable agricultural development: A review. Ournal of Pharmacognosy and Phytochemistry, 7: 1915–1921.

Li Y, Zhang J, Zhang J, Xu W, Mou Z. 2019. Characteristics of Inorganic Phosphate-Solubilizing Bacteria from the Sediments of a Eutrophic Lake. Int. J. Environ. Res. Public Health, 16: 1-15 https://doi.org/10.3390/ijerph16122141

Marpaung AE, Hanum H, Sembiring M. 2021. The effect of liquid organic fertilizer and phosphate solubilising bacteria Bacillus sp on potato growth (*Solanum tuberosum*) in andisol soil. IOP Conference Series: Earth and Environmental Science, 807: 1-6. https://doi.org/10.1088/1755-1315/807/4/042079

Marpaung AE, Sabrina T, Rauf A, Susilowati DN. 2024a. Microbial phosphate solubilization mechanisms in P solubilizing in andisol. Kultivasi, 23: 227-238. https://doi.org/10.24198/kultivasi.v23i2.52749

Marpaung AE, Sabrina T, Rauf A, Susilowati DN. 2024b. The role of phosphate solubilizing fungi from the shallot rhizosphere on increasing the growth and yield of shallot on Andisol. IOP Conference Series: Earth and Environmental Science, 1417: 1-9.

https://doi.org/10.1088/1755-1315/1417/1/012003

Mehta P, Walia A, Chauhan A, Shirkot CK. 2013. Plant growth promoting traits of phosphate-solubilizing rhizobacteria isolated from apple trees in trans Himalayan region of Himachal Pradesh. Archives of Microbiology, 195: 357–369. https://doi.org/10.1007/s00203-013-0881-y

Kishore N, Pavan K, Pindi, Ram Reddy S. 2015. Phosphate-Solubilizing Microorganisms: A Critical Review. Plant Biology and Biotechnology: Plant Diversity, Organization, Function and Improvement, 1: 1–827. https://doi.org/10.1007/978-81-322-2286-6

Perea Rojas Y del C, Arias RM, Medel Ortiz R, Trejo Aguilar D, Heredia G, Rodríguez Yon Y. 2019. Effects of native arbuscular mycorrhizal and phosphate-solubilizing fungi on coffee plants. Agroforestry Systems, 93: 961–972. https://doi.org/10.1007/s10457-018-0190-1

Setiawati MR, Pranoto E. 2015. Perbandingan Beberapa Bakteri Pelarut Fosfat Eksogen Pada Tanah Andisol Sebagai Areal Pertanaman Teh Dominan Di Indonesia. Jurnal Penelitian Teh Dan KIna, 18: 159–164. https://www.researchgate.net/publication/307575626

Sharma R, Walia A, Chauhan A, Shirkot CK. 2015. Multi-trait plant growth promoting bacteria from tomato rhizosphere and evaluation of their potential as bioinoculants. Applied Biological Research, 17: 113-124. https://doi.org/10.5958/0974-4517.2015.00019.1

Silva LI da, Pereira MC, Carvalho AMX de, Buttrós VH, Pasqual M, Dória J. 2023. Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. Agriculture, 1: 1-30. MDPI. https://doi.org/10.3390/agriculture13020462

Sriwantoko S, Syam'un E, Ulfa F. 2020. Growth of red onion plant (*Allium ascalonicum* L.) in an application by phosphate solubilizing microbes and goat dung compost. Advances in Environmental Biology, 14: 23–30. https://doi.org/10.22587/aeb.2020.14.7.4

Sukarman, Dariah A. 2014. Andosol Soil in Indonesia: Characteristics, Potential, Constraints, and Management for Agriculture. In Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian, Kementerian Pertanian (Issue 12).

Sukarman, Dariah A. 2015. Tanah Andosol di Indonesia. In Balai Besar Penelitian dan Pengembangan Sumberdaya Lahan Pertanian, Kementerian Pertanian (Issue 12).

Takahashi, T., & Dahlgren, R. A. (2016). Nature, properties and function of aluminum–humus complexes in volcanic soils. *Geoderma*, 263: 110-121. https://doi.org/10.1016/j.geoderma.2015.08.032

Tian J, Ge F, Zhang D, Deng S, Liu X. 2021. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical p cycle. Biology, 10: 1–19. https://doi.org/10.3390/biology10020158

Timofeeva A, Galyamova M, Sedykh S. 2022. Prospects for Using Phosphate-Solubilizing Microorganisms as Natural Fertilizers in Agriculture. In Plants, 11: 1-23. MDPI. https://doi.org/10.3390/plants11162119

Wang Q, Ding J, Xie H, Hao D, Du Y, Zhao C, Xu F, Kong Q, Wang B. 2021. Phosphorus removal performance of microbial-enhanced constructed wetlands that treat saline wastewater. Journal of Cleaner Production, 288, 125119. https://doi.org/10.1016/J.JCLEPRO.2020.125119

Zhu J, Li M, Whelan M. 2018. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. Science of The Total Environment, 612, 522–537. https://doi.org/10.1016/J.SCITOTENV.2017.08.095

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Riyanti A · Lakitan B · Imanudin MS · Yazid M

Estimating leaf area in velvetleaf (*Limnocharis flava*) and kangkong (*Ipomea aquatica*): a precise and non-destructive approach for wetland vegetables

Abstract. Developing a leaf-area estimation model for vegetable cultivars in wetlands is essential to optimizing agricultural cultivation practices. This study aims to develop a non-destructive model for leaf area estimation in wetland vegetable cultivars (velvetleaf (*Limnocharis flava*) and kangkong (*Ipomoea aquatica*)) using regression-based models. The plants were cultivated in a wetland system. Measurements of leaf length and width were taken on all leaves of each plant, using the product of length and width (L×W) as predictor. The regression models for estimating leaf area were adjusted from linear, zero-intercept linear, quadratic, and power. The optimal model was evaluated using the determination coefficient (R^2) and the Root Mean Square Error (RMSE). The results showed that the most reliable regression model for estimating velvetleaf leaf area was linear regression with the equation y = 0.881LW - 7.615 (R^2 = 0.954; RSME = 7.916), and the power model for kangkong leaf area, with the equation y = $0.9407LW^{0.9309}$ (R^2 = 0.970; RSME = 1.695). Differences in leaf shape among plant species result in different accuracies of leaf area estimation models. Thus, the model should be useful to guide future research and practical applications in monitoring leaf growth and determining harvest time.

Keywords: Leaf area estimation · Leaf shape · Regression models · Vegetable crops · Wetlands

Submitted: 20 January 2026, Accepted: 27 April 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69353

Riyanti A[1,4*] · Lakitan B[2,3] · Imanudin MS[2] · Yazid M[2]
[1]Graduate School of Enviromental Science, Universitas Sriwijaya, Palembang 30139, South Sumatra, Indonesia
[2]Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30662, South Sumatra, Indonesia
[3]Research Center for Sub-optimal Lands, Universitas Sriwijaya, Palembang 30139, South Sumatra, Indonesia
[4]Department of Environmental Engineering, Faculty of Engineering, Universitas Batanghari, Jambi 36122, Indonesia
*Correspondence: anggrika.riyanti@unbari.ac.id

Riyanti A, Lakitan B, Imanudin MS, Yazid M. 2026. Estimating leaf area in velvetleaf (*Limnocharis flava*) and kangkong (*Ipomea aquatica*): a precise and non-destructive approach for wetland vegetables. Jurnal Kultivasi, 25 (1): 44-53.

Introduction

The current rise in public awareness of the importance of vegetable consumption in promoting health has significantly increased the demand for vegetables in Indonesia. Vegetable and fruit consumption in Indonesia in 2024 reached around 89.3 kg per capita per year, equivalent to 244 grams per day (Ministry of Agriculture, 2024). This number is still far below the WHO recommendation of a minimum consumption of 400 g/day of fruits and vegetables (Ruiz-López & García-Villanova, 2023). The situation increases the opportunity for the development of domestic vegetable production.

The use of wetlands for vegetable cultivation can be a strategy for diversifying agricultural businesses while optimizing underutilized land. Indonesia has an estimated 20.6 to 40.5 million hectares of wetlands spread across various regions, mainly in Sumatra, Kalimantan, and Papua (Sulaiman et al., 2019). Approximately 9.53 million hectares of wetlands in Indonesia have potential for agricultural use, of which 6 million hectares are suitable for food crops (Aristin et al., 2024).

The use of wetlands for vegetable cultivation can be a strategy for diversifying agricultural businesses while optimizing underutilized land. Based on the inventory data from the Ministry of Environment and Forestry, Indonesia has an estimated 20.6 million hectares of wetlands spread across various regions in Sumatra, Kalimantan, and Papua (Bappenas, 2023). The largest portions of these wetlands consist of 13.43 million hectares of peatlands and 3.45 million hectares of mangroves. Approximately 9.53 million hectares of wetlands in Indonesia have potential for agricultural use, of which 6 million hectares are suitable for food crops (Aristin et al., 2024).

One important aspect of developing vegetables in wetlands is understanding plant growth characteristics, particularly leaf area. Leaf area is a very important physiological parameter due to its fundamental role in plant growth, photosynthates, and plant productivity (Rahimikhoob et al., 2023). Leaf area affects the efficiency of sunlight absorption and the production of photosynthates that will be translocated to plant organs (Pandey et al., 2021). In wetland conditions with specific environmental characteristics, information on the leaf area of vegetable plants is crucial for understanding plant growth responses to environmental stresses such as high humidity, low pH, and limited oxygen availability in the root zone.

Accurate leaf area measurement is necessary for various research and cultivation purposes, including plant growth analysis, leaf area index calculation, net assimilation rate, and evaluation of plant response to cultivation treatments (Dias et al., 2022). However, conventional leaf area measurement using destructive methods, such as gravimetry or millimeter paper, is time-consuming, costly, and damaging to the plants being observed (Ribeiro et al., 2019). Therefore, the current development of leaf area estimation methods is a leaf-dimension-based method (leaf length and width) that uses certain leaf shape constants or coefficients (Kumar et al., 2022). This method has advantages because it is non-destructive, easy to do, fast and accurate, does not require expensive equipment, and can be applied directly in the field. This non-destructive method is particularly important for long-term research that requires repeated observations on the same plants.

Estimating leaf area using non-destructive methods, particularly through linear regression, has emerged as an essential approach in agricultural and botanical research. Non-destructive techniques serve various applications, including assessing plant health, monitoring photosynthetic efficiency, and optimizing resource management under varying environmental conditions. The resulting model can be used to monitor leaf development by measuring leaf length and width without picking the leaves. By offering a low-cost, effective alternative to destructive methods, they enable researchers to repeatedly measure leaf area, allowing time-series data to be collected on the same leaves without disrupting their growth (Dias et al., 2022; Madhavi et al., 2022).

Several studies have demonstrated the efficacy of linear regression models in estimating leaf area from easily measurable parameters such as leaf length and width. Pacheco et al. (2020) illustrate how simple linear regression can yield high coefficients of determination (>0.90) when applied to leaf morphological dimensions, establishing a solid foundation for predicting leaf area. Similarly, Kumar et al. (2022) emphasize the advantages of non-destructive methods in approximating leaf area, specifically in the context of evaluating photosynthetic capacity and growth metrics. These findings are confirmed by Lakitan et al. (2023), who found that a linear relationship between leaf area and lamina dimensions is preferable for rapid measurements.

Despite these advancements, a notable gap remains in applying these techniques across diverse crop species and environmental conditions. While existing models may perform effectively for specific plants, their reliability across a broader range of species remains to be validated. Furthermore, Sabr (2020) points out that many existing models may not adequately account for variability introduced by different ecological contexts, indicating a need for the development of more universally applicable models. Enriching datasets to cover a broader range of species and environmental conditions is essential to enhance the applicability of non-destructive methods. The linear models of leaf area are specific to each plant species and can be influenced by growing conditions (Oliveira et al., 2019).

Current approaches have evolved beyond simple linear regression to incorporate more complex methods that improve accuracy, depending on leaf shape and plant species. The most common approaches use the product of length (L) and width (W), including methods with a zero intercept, power functions, and polynomials (Lakitan et al., 2023; Muda et al., 2024; Ribeiro et al., 2022). Muda et al. (2024) found that zero-intercept regression was suitable for estimating leaf area in butterhead lettuce grown under shallow water tables. The selection of a more accurate model is based not only on the R^2 value but also on the lowest RMSE, which indicates a smaller average prediction error.

At present, research on leaf area estimation in vegetable crops cultivated in wetlands remains very limited, resulting in a lack of accurate, reliable models for the specific wetland conditions in Indonesia. Information on leaf area is essential for understanding how vegetable crops grow in specific environmental conditions and for optimizing cultivation practices. This study observes two wetland-based vegetable crops, velvetleaf and kangkong, which are widely cultivated in Indonesia. Kangkong is ranked among the top five most consumed vegetables by the Indonesian population (Statistics Indonesia, 2025), while velvetleaf is a vegetable currently widely cultivated as a commercial commodity (Ramagita et al., 2025).

Unlike many dryland vegetables that possess relatively stable leaf geometries, velvetleaf and kangkong exhibit the physical capacity to adjust their morphology, such as stem length and leaf width, to survive in their respective environments. Kangkong frequently undergoes a transition from a narrow-lanceolate to a broad-cordate leaf shape, while velvetleaf adjusts its blade-to-petiole ratio in response to water depth. Consequently, general linear models often fail to capture these non-linear allometric shifts.

Therefore, this study aims to develop a regression model to estimate leaf area for velvetleaf and kangkong that can be used practically, accurately, and efficiently, accounting for morphological growth and leaf shape variation in vegetable crops. This study addresses the gap by comparing several regression models, including linear, zero-intercept, quadratic, and power regression approaches, ensuring a model that remains accurate across diverse growth stages under wetland environmental conditions. With a reliable model, leaf area measurements can be conducted more easily and quickly, supporting various research and applications in wetland agriculture, while helping farmers and field practitioners measure leaf area without compromising accuracy. A precise leaf-area estimation model is expected to fill the existing knowledge gap and provide practical contributions to vegetable crop management in wetland ecosystems.

Materials and Methods

The research was carried out during the rainy season from February to March 2025 with a tropical climate. The experimental site was a lowlands urban area, located in Pematang Sulur Village, Telanaipura, Jambi City. Temperature and humidity measurements during the study are shown in Fig. 1. The plants used in this study were vegetables commonly sold in markets and consumed by the community, namely velvetleaf and kangkong. Both plants can survive in waterlogged soil and have different leaf shapes. Velvetleaf leaves are oval-shaped, smooth, and have curved veins. Meanwhile, kangkong has arrowhead or heart-shaped leaves with pointed tips and a smooth surface.

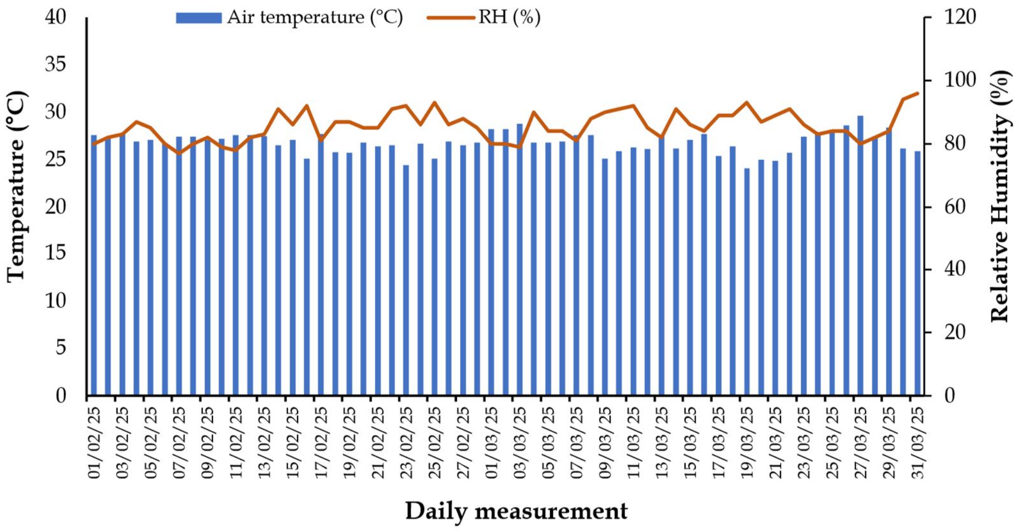


Figure 1. Daily air temperature and relative humidity during the research

The velvetleaf used in this study was derived from certified seeds. Velvetleaf seeds were germinated by sowing them in a container filled with a mixture of soil and water until sprouts appeared (approximately 30 days), then transferred to the nursery. Meanwhile, kangkong was propagated vegetatively using 10 cm-long stem shoots, then planted in a nursery containing a 1:1 mixture of soil and manure and watered until the soil was moist. After 2 weeks, each plant was transferred to a plastic pot containing 2 kg of soil. Then every pot was filled with 400 ml of water until the soil was flooded up to the lower stem, approximately 5 cm above the soil surface. The plants were placed in an area covered with a transparent plastic roof to prevent rainwater from entering, but still exposed to sunlight. To maintain waterlogged conditions, water was added daily until the water level reached 5 cm above the soil surface. Plants were fertilized by spraying Bayfolan D leaf fertilizer at 2 mL L^{-1} every 4 days.

Data collection. The study was conducted with three replicates. Each group consisted of 32 plants, with 16 plants of each type, yielding a total of 48 plants of each type. The total number of plants (population) was 96, and 50% were randomly selected as samples. Leaf length (L) and leaf width (W) were measured on all leaves of an individual plant, starting when the leaves began to open (Fig 2). Observations were conducted every two days over an 18-day period, starting from 28 days after planting at the onset of the vegetative phase until 46 days after planting at the end of the vegetative stage, marked by the emergence of flowers. Leaf length was measured along the midrib, starting from the base of the leaf (the point where the leaf blade meets the petiole) to the tip of the leaf. Leaf width was measured at the widest part of the leaf blade, from the left edge to the right edge. Immature leaves were measured when they had fully unfolded.

Leaf area (LA) was predicted using the product of length and width (L×W) as the predictor in a simple regression model. The predicted leaf area was verified by comparing the regression values and actual leaf area measurements. Actual leaf area was measured at harvest by collecting all leaves from each sample plant and measuring them individually using a Digital Image Scanner with Easy Leaf Area software. This software is used for quick and non-destructive leaf area measurements by placing leaf samples on a white background and taking digital photos (Easlon & Bloom, 2014). The number of leaf samples in this study was 200–400, which represents a strong sample size (n > 200) to ensure the model has statistical power and represents population variability while remaining accurate across various growth stages (Pérez-Harguindeguy et al., 2013).

Data analysis. Various regression models have been used in previous studies to predict leaf area. This study used several of the most accurate regression models to predict leaf area, namely: (a) linear model, (b) zero-intercept linear, (c) quadratic, and (d) power (Lakitan et al., 2023;

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Muda et al., 2024). The predictor used to predict leaf area in this study was L×W. The L×W predictor is the most optimal variable for predicting leaf area in plant species with different leaf shapes (Lakitan et al., 2017; Liu et al., 2017) and provides smaller RMSE results compared to regression models with predictors L and W (Ribeiro et al., 2024). The most accurate regression model was evaluated based on the coefficient of determination (R^2) dan the lowest RMSE. All statistical analyses were performed using IBM SPSS Statistics Version 25 for Windows (IBM Corporation, Armonk, USA) with a significance level of $p < 0.05$.

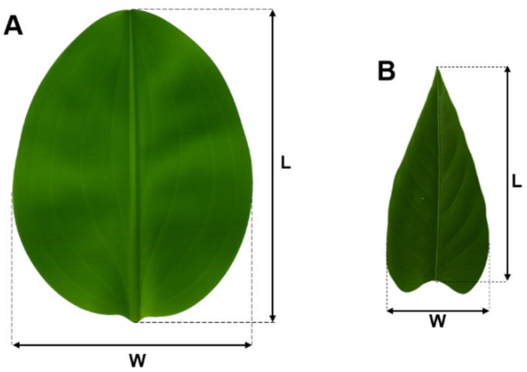


Figure 2. Illustrative of leaf midrib length (L) and width (W) in velvetleaf (A) and kangkong (B)

Results and Discussion

The area of the main leaf was determined by the length and width of the leaf, so the leaf growth observed in this study focused on the increase in leaf blade length and leaf width. Differences in plant species affect the rate of growth and development of leaves between plants. Table 1 displays the descriptive statistics for length, width, and aspect ratio (L/W) data along with the maximum, minimum, mean, and standard deviation. Leaf length was measured after the leaves had fully opened. The length of velvetleaf leaf blades ranged from 16.8 to 5.9 cm with an average of 11.21 cm and an average leaf elongation of 18 mm. Meanwhile, leaf width ranged from 15.5 to 4.7 cm with an average of 9.6 cm and an average leaf expansion of 13 mm. The increase in length and width of velvetleaf leaves occurred more rapidly in the early observation period until day 4, then increased slowly with the addition of new leaves until day 18 (Fig. 3).

The area of the main leaf was determined by the length and width of the leaf, so the leaf growth observed in this study focused on the increase in leaf blade length and leaf width. Differences in plant species affect the rates of leaf growth and development. Table 1 presents descriptive statistics for length, width, and aspect ratio (L/W), including maximum, minimum, mean, and standard deviation. Leaf length was measured after the leaves had fully opened. The length of velvetleaf leaf blades ranged from 16.8 to 5.9 cm, with an average of 11.21 cm and an average leaf elongation of 18 mm. Meanwhile, leaf width ranged from 15.5 to 4.7 cm with an average of 9.6 cm and an average leaf expansion of 13 mm. The increase in length and width of velvetleaf leaves occurred more rapidly during the early observation period, from day 0 to day 4, then increased slowly with the addition of new leaves until day 18 (Fig. 2).

The increase in length and width of velvetleaf leaves is shown in Figure 3 (A and B). Velvetleaf leaves showed more consistent growth at each observation, with an average increase in length of 1.5 cm over the observation period. Meanwhile, the average increase in leaf width was greater than the increase in leaf length, which was 2.5 cm during the observation period.

Table 1. Descriptive analysis of leaf length (L) and width (W) in velvetleaf and kangkong

Plant	Parameter	Maximum	Minimum	Mean ± standard deviation
Velvetleaf	Length (cm)	16.80	5.90	11.21 ± 0.36
	Width (cm)	15.50	4.70	9.60 ± 0.49
	L/W ratio	1.33	1.17	1.23 ± 0.05
Kangkong	Length (cm)	12.6	1.9	6.95 ± 0.44
	Width (cm)	6.4	1.1	3.22 ± 0.23
	L/W ratio	2.24	2.00	2.09 ± 0.09

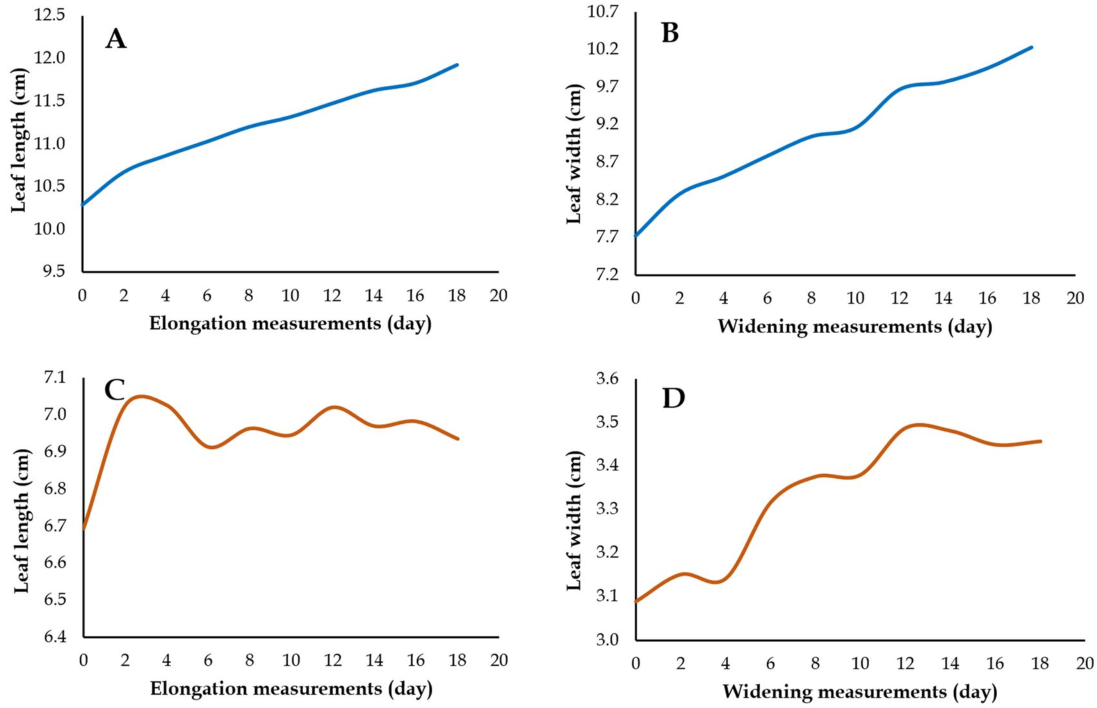


Figure 3. Daily leaf elongation and widening of velvetleaf (A and B) and kangkong (C and D)

Meanwhile, the growth of kangkong leaves is shown in Figure 3 (C and D). The growth graph shows that the average increase in the length and width of kangkong leaves is highly variable and inconsistent across observations. The increase in the length of kangkong leaves is faster in the first 4 days, but tends to be stable (no increase in leaf length) until the end of the observation. The average increase in kangkong leaf length was 0.2 cm. Meanwhile, the increase in kangkong leaf width was greater during the observation period, averaging 0.4 cm. This value indicates that leaf growth in both plants experienced a greater increase in leaf width than in leaf length.

Meanwhile, the ratio of leaf length to width is used to predict leaf shape. A higher L/W ratio indicates a more slender/elongated leaf shape, while a ratio of less than 1 indicates a more symmetrical leaf shape (Lakitan et al., 2023). Figure 4 shows that leaf shape development during the observation period was relatively consistent. The average L/W ratio for velvetleaf leaves was 1.23, while the L/W ratio for kangkong leaves was 2.09, twice that of velvetleaf leaves. This value is consistent with the slender shape of kangkong leaves with elongated tips. Meanwhile, the L/W ratio value for velvetleaf leaves was close to 1, indicating oval-shaped leaves.

Leaf shape is commonly used as the main characteristic in identifying plant species and is often considered a constant trait. Leaf growth has a self-regulatory pathway but is also influenced by increases in cell number, growth conditions, environmental factors, and plant genetics (Vercruysse et al., 2020). Leaf growth has a specific time limit, marked by the cessation of cell division earlier at the leaf tip and later at the leaf base. Once cell division stops, the rate of leaf growth will cease (Lakitan et al., 2018). There are differences in leaf size when they begin to open; velvetleaf leaves reach their full size in about 2 days, while kangkong leaves take about 8 days. In some plant species, leaf shape can change during the development stage (Li et al., 2020). However, in this study, the length and width ratio of velvetleaf and kangkong leaves during growth did not undergo significant changes, indicating that the shape of velvetleaf and kangkong leaves remained static/constant during the leaf enlargement process.

Regression model for leaf area prediction. Regression models and equations were obtained from the relationship between actual leaf area measurements and leaf length and width. In this study, the predictor used was L×W because it achieved the highest accuracy and the lowest

RMSE compared to using L and W separately (Irwan & Wicaksono, 2017; Ribeiro et al., 2024). Leaf area testing used four regression models, namely linear, zero-intercept linear, quadratic, and power. Model accuracy was based on the higher coefficient of determination (R^2) and the lowest RMSE. However, in the context of model prediction accuracy, selecting an accurate model prioritizes absolute error metrics, such as RMSE (Ribeiro et al., 2022) . A lower R^2 value does not necessarily mean that the model is less accurate.

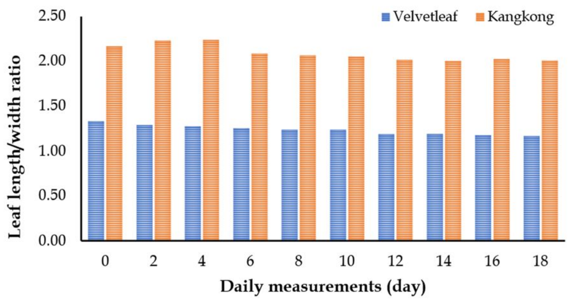


Figure 4. Ratio of leaf length and width (L/W) in velvetleaf and kangkong

Based on the results in Table 2, the most accurate model for estimating leaf area was the linear model for velvetleaf, and the power model for kangkong resulted in R^2 of 0.954 and 0.970, and RMSE of 7.916 and 1.695 for velvetleaf and kangkong, respectively. This model was selected based on the lowest RMSE, indicating a smaller average prediction error. Thus, the reliable equations to estimate leaf area for velvetleaf and kangkong are $y = 0.881LW - 7.615$ and $y = 0.9407LW^{0.9309}$, respectively. These results are similar to previous studies, which showed that the linear regression model using the LW predictor is effective and accurate for estimating leaf area with simple shapes across multiple species (Muda et al., 2024; Ribeiro et al., 2022). Meanwhile, the power model is occasionally more accurate for species with complex leaf shapes (Ribeiro et al., 2019). The differences in the selected models between velvetleaf and kangkong are due to differences in leaf shape, for which the linear model is more appropriate for predicting the area of oval-shaped and symmetrical leaves. In contrast, the power regression is more appropriate for slender leaves with tapered tips. A significant relationship between predicted and actual leaf area was confirmed using the selected model, as shown in Figure 5.

The total leaf area development in each subsequent observation was predicted using the selected regression equation model. The development of velvetleaf leaf area increased until day 12, then began to decline until day 18 (Fig. 6A). This occurred because some older leaves began to yellow and die, and the addition of new leaves slowed and then stopped. Meanwhile, in kangkong, the total leaf area continued to increase until day 18. It indicates that in kangkong, new leaves grow in greater numbers and more rapidly than in velvetleaf (Fig. 6B).

Based on the above discussion, accurate determination of leaf length and width is necessary to estimate leaf area for velvetleaf and kangkong to support vegetable production. The data reported in this study are consistent with previous research on other species, which found that model validation using a combination of measured leaf length and width yielded a high correlation between observed and predicted leaf area (Muda et al., 2024; Ribeiro et al., 2024). Under the same environmental conditions, namely in a wetland environment, research by Lakitan et al., (2023) on three varieties of Swiss chard showed that multiplying the length and width of the leaves provided an accurate estimate of leaf area. Thus, the results of this study indicate that the most reliable regression models for estimating leaf area in velvetleaf and kangkong were linear and power, respectively, using the LxW predictor.

Table 2. Regression model for predicting leaf area using LxW as predictor

Vegetable	Regression type	Equation	R^2	RSME
Velvetleaf	Linear	LA = 0.881LW - 7.615	0.954	7.916
	Zero-intercept linear	LA = 0.828LW	0.995	8.231
	Quadratic	LA = $0.0005(LW)^2$ + 0.763LW - 0.651	0.955	7.938
	Power	LA = $0.599LW^{1.064}$	0.964	21.234
Kangkong	Linear	LA = 0.697LW + 1.304	0.969	1.722
	Zero-intercept linear	LA = 0.74LW	0.993	1.805
	Quadratic	LA = $-0.002(LW)^2$ + 0.798LW + 0.231	0.970	1.710
	Power	LA = $0.9407LW^{0.9309}$	0.970	1.695

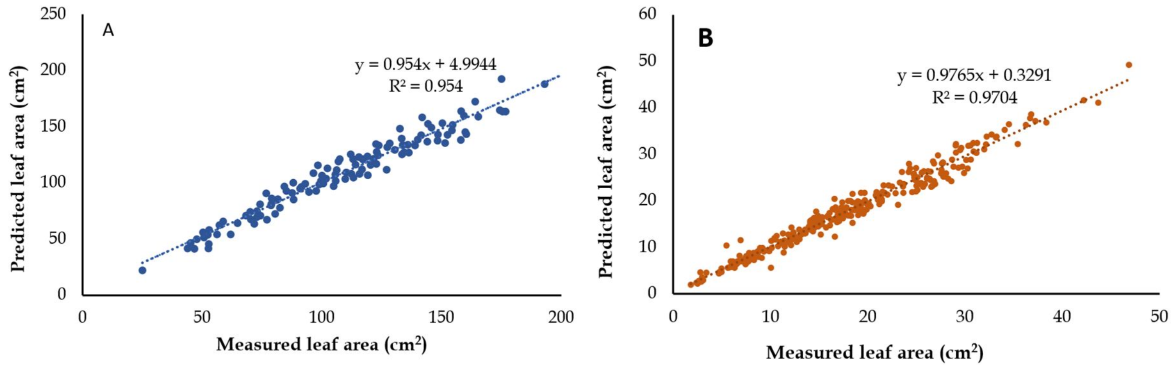


Figure 5. The relationship between predicted and measured leaf area (LA) regression model for velvetleaf (A) and kangkong (B)

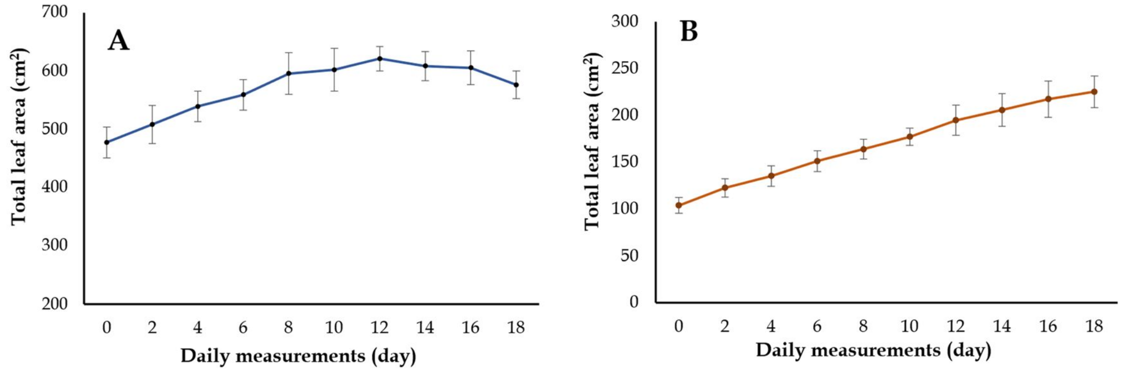


Figure 6. Total leaf area of velvetleaf (A) and kangkong (B) using zero-intercept linear models

Conclusion

The linear regression model developed in this study provides a practical, non-destructive solution for estimating leaf area. However, it remains limited to specific plant species and environmental conditions. Since leaf shape can vary depending on growth conditions, this formula may require further adjustment when applied under different conditions. For future research, it is essential to test these models across various land types and leverage technologies such as machine learning (artificial intelligence). By integrating manual methods with machine learning, more adaptive models can be developed to account for structural changes in plants across varying environments, thereby achieving higher accuracy without damaging field samples.

Acknowledgments

The authors gratefully thank the Indonesian Ministry of Higher Education, Science, and Technology for the financial support provided for the research.

References

Aristin NF, Taryana D, Ruja IN. 2024. Wetland Studi Kasus Dinamika Wilayah Banjarmasin. Media Nusa Creative (MNC Publishing).

Bappenas. 2023. Laporan Kajian Lingkungan Hidup Strategis RPJPN 2025-2045. Ministry of National Development Planning.

Dias MG, Mela D, Silva TI da, Ribeiro JE da S, Grossi JAS, Zuin AHL, Martinez ACP, Barbosa JG. 2022. Leaf area estimation of Congea tomentosa using a non-destructive method. Revista Brasileira de Engenharia Agrícola e Ambiental, 26(10), 729–734. https://doi.org/https://doi.org/10.1590/1807-1929/agriambi.v26n10p729-734

Easlon HM, Bloom AJ. 2014. Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. Applications in Plant Sciences, 2(7), 1400033. https://doi.org/https://doi.org/10.3732/apps.1400033

Irwan AW, Wicaksono FY. 2017. Comparations of soybean's leaf area measurement using gravimetry, regression, and scanning. https://doi.org/doi.org/10.24198/kultivasi.v16i3.14448

Kumar B, Mahto RK, Doss SG, Aparna K, Yadav H. 2022. Estimation of single leaf area in Major Tasar Host Plant Species (Arjun, Asan and Jarul) through non-destructive method. Plant Archives, 22((Spl. Issue (VSOG))), 260–264. https://doi.org/https://doi.org/10.51470/plantarchives.2022.v22.specialissue.047

Lakitan B, Alberto A, Lindiana L, Kartika K, Herlinda S, Kurnianingsih A. 2018. The benefits of biochar on rice growth and yield in tropical riparian wetland, South Sumatra, Indonesia. Chiang Mai University Journal of Natural Sciences, 17(2), 111–126. https://doi.org/10.12982/CMUJNS.2018.0009

Lakitan B, Ilman Widuri L, Meihana M. 2017. Simplifying procedure for a non-destructive, inexpensive, yet accurate trifoliate leaf area estimation in snap bean (Phaseolus vulgaris). In Journal of Applied Horticulture (www.horticultureresearch.net) Journal of Applied Horticulture (Vol. 19, Issue 1). https://doi.org/https://doi.org/10.37855/jah.2017.v19i01.03

Lakitan B, Susilawati S, Wijaya A, Ria RP, Muda SA. 2023. Leaf blade growth and development in Red, Pink, and Yellow petiole cultivars of the Swiss Chards grown in floating culture system. Jordan Journal of Biological Sciences, 16(1)(1), 157–164. https://doi.org/https://doi.org/10.54319/jjbs/160119

Liu Z, Zhu Y, Li F, Jin G. 2017. Non-destructively predicting leaf area, leaf mass and specific leaf area based on a linear mixed-effect model for broadleaf species. Ecological Indicators, 78, 340–350. https://doi.org/10.1016/j.ecolind.2017.03.025

Li Y, Zou D, Shrestha N, Xu X, Wang Q, Jia W, Wang Z. 2020. Spatiotemporal variation in leaf size and shape in response to climate. Journal of Plant Ecology, 13(1), 87–96. https://doi.org/https://doi.org/10.1093/jpe/rtz053

Madhavi BGK, Bhujel A, Kim NE, Kim HT. 2022. Measurement of overlapping leaf area of ice plants using digital image processing technique. Agriculture (Switzerland), 12(9)(9), 1321. https://doi.org/10.3390/agriculture12091321

Ministry of Agriculture. 2024. Statistics of Food Consumption.

Muda S, Lakitan B, Ramadhani F. 2024. Butterhead lettuce growth under shallow water tables and its recovery on tropical urban ecosystem. https://doi.org/10.36253/ahsc16233

Oliveira VDS, Gonçalves LC, Costa A, Dos Santos KTH, Santos JSH, Santos GP, Chisté H, Schmildt O, Czepak MP, da Vitória EL. 2019. Mathematical modeling to estimation leaf area of Pimenta dioica from linear dimensions. International Journal of Plant & Soil Science, 28(2), 1–8. https://doi.org/https://doi.org/10.9734/ijpss/2019/v28i230104

Pacheco AB, Nascimento JG, Moura LB, Lopes TR, Duarte SN, Coelho RD, Marques PAA. 2020. Non-destructive and destructive methods to determine the leaf area of Zucchini. J. Agric. Stud, 8(3), 295. https://doi.org/https://doi.org/10.5296/jas.v8i3.16299

Pandey AK, Singh AG, Gadhiya AR, Kumar S, Singh D, Mehta R. 2021. Current approaches in horticultural crops to mitigate waterlogging stress. Stress Tolerance in Horticultural Crops, 289–299. https://doi.org/https://doi.org/10.1016/B978-0-12-822849-4.00014-0

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, De Vos AC, Cornelissen JHC. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany, 61(3), 167–234. https://doi.org/10.1071/BT12225

Rahimikhoob H, Delshad M, Habibi R. 2023. Leaf area estimation in Lettuce: comparison of Artificial Intelligence-based methods with image analysis technique. Measurement, 222, 113636. https://doi.org/https://doi.org/10.1016/j.measurement.2023.113636

Ramagita MA, Juan J, Putra Y, Kasmiyati S, Kristiani BE. 2025. Dualisme Pemanfaatan Gulma Genjer (Limnocharis flava): Peluang Sebagai Agen Fitoremediasi dan Pangan Nutraseutika Dual Utilization of Genjer ' s Weed (Limnocharis flava): Opportunities as a Phytoremediation Agent and Nutraceutical Food Source Pendahul. 10(2), 191–203. https://doi.org/10.24002/biota.v10i2.10270

Ribeiro JE da S, Côelho E dos S, Lopes W de AR, Silva EF da, Oliveira AKS de, Oliveira PH de A, Silva AGC da, Jardim AM da RF, Silva DV, Barros AP. 2024. Allometric equations to predict the leaf area of castor bean cultivars. Ciência Rural, 55(1), e20230550. https://doi.org/https://doi.org/10.1590/0103-8478cr20230550

Ribeiro JE da S, dos Santos Coêlho E, Figueiredo FRA, Pereira WE, de Albuquerque MB. 2019. Leaf area estimation for Psychotria carthagenensis and Psychotria hoffmannseggiana as a function of linear leaf dimensions. Acta Scientiarum. Biological Sciences, 41, 43494. https://doi.org/https://doi.org/10.4025/actascibiolsci.v41i1.43494

Ribeiro JE da S, Romário Andrade Figueiredo F, Silva Nóbrega J, dos Santos Coêlho E, Ferreira Melo M. 2022. LEAF AREA OF Erythrina velutina Willd. (FABACEAE) BY USING ALLOMETRIC EQUATIONS. https://doi.org/10.5380/rf.v52

Ruiz-López MD, García-Villanova B. 2023. Fruits and Vegetables. In B. Caballero (Ed.), Encyclopedia of Human Nutrition (Fourth Edition) (pp. 397–411). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-821848-8.00124-4

Sabr H. 2020. Prediction of leaf area by a non-destructive method of Platanus orientalis tree. Journal of University of Duhok, 23(2), 211–217. https://doi.org/https://doi.org/10.26682/ajuod.2020.23.2.24

Statistics Indonesia. 2025. Statistik Hortikultura. Badan Pusat Statistik.

Sulaiman AA, Sulaeman Y, Minasny B. 2019. A framework for the development of wetland for agricultural use in Indonesia. *Resources*, *8(1)*(1), 34. https://doi.org/https://doi.org/10.3390/resources8010034

Vercruysse J, Baekelandt A, Gonzalez N, Inzé D. 2020. Molecular networks regulating cell division during Arabidopsis leaf growth. *Journal of Experimental Botany*, *71*(8), 2365–2378. https://doi.org/doi.org/10.1093/jxb/erz522

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Sistyananda FN · Suminar E · Nuraini A · Kadapi M · Murgayanti · Mubarok S · Budiarto R · Renaldi E · Kusumiyati

Optimization of *Physalis angulata* L. callus induction and salinity-induced antioxidant production

Abstract. Groundcherry (*Physalis angulata* L.) is a plant with many medicinal potentials due to its rich secondary metabolites such as phenolic and flavonoid. However, conventional agriculture practices are still limited, especially in Indonesia. This study was divided into two stages. The first stage was conducted to determine optimal 6-BAP and 2,4-D combination for callus induction, while the second stage was conducted to determine callus phenolic, flavonoid, and antioxidant response to salinity stress. The first stage was arranged in factorial completely randomized design with two factors: 6-BAP (0, 2, and 4 mg/L) and 2,4-D (0, 0.5, 1, and 1.5 mg/L). The second stage was arranged in simple completely randomized design with different NaCl concentration (0, 25, 50, 75, 100 mM) as treatments. The results showed significant interaction ($p < 0.05$) between 6-BAP and 2,4-D on callus induction. Combination of 2 mg/L 6-BAP and 1 mg/L 2,4-D showed the highest callus formation percentage (46% increase), callus size (60.12% increase), and fresh weight (179.69% increase), and greener compact callus. Application of NaCl as salinity stress at second stage experiment served as an elicitor to enhance callus antioxidant capacity. Salinity level at 100 mM NaCl showed the most accumulation of phenolic content (17.8% increase), flavonoid content (25.17% increase), and antioxidant activities (6.84% IC_{50} decrease). This study demonstrates plant growth regulator optimization with salinity stress elicitation integration as an effective strategy to enhance antioxidant production in *P. angulata* callus, providing a practical approach for controlled secondary metabolite production.

Keywords: Flavonoid · Groundcherry · Phenolic content · Salinity stress · Secondary metabolites

Submitted: 28 January 2026, Accepted: 31 March 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69556

Sistyananda FN*[1] · Suminar E[2] · Nuraini A[3] · Kadapi M[3] · Murgayanti[2] · Mubarok S[2] · Budiarto R[2] · Renaldi E[4] · Kusumiyati[3]
[1]Agrotechnology Student, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
[2]Agrotechnology Study Program, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
[3]Agronomy Study Program, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
[4]Agribusiness Study Program, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
*Correspondence: firstian21001@unpad.ac.id

Sistyananda FN, Suminar E, Nuraini A, Kadapi M, Murgayanti, Mubarok S, Budiarto R, Renaldi E, Kusumiyati. 2026. Optimization of *Physalis angulata* L. callus induction and salinity-induced antioxidant production. Jurnal Kultivasi, 25 (1): 54-67.

Introduction

Groundcherry is one of the medicinal plants that grows in Indonesia. *P. angulata* can thrive in various places such as rice fields, gardens, forests, and even roadsides. However, this plant becomes harder to find because it is often considered as a weed and eradicated by many farmers (Nuranda et al., 2016). Any research and agricultural practices in Indonesia are relatively narrow and limited unlike Brazil and Mexico (da Silva Ramos et al., 2021; Vargas-Ponce et al., 2016). *P. angulata* has been widely used as a raw material in biopharmaceutical and non-biopharmaceutical products in various regions due to its many benefits. *P. angulata* has many potentials as antioxidant, anti-inflammation, anticancer, antibacterial, antidiabetic, and antidiarrheal agent due to its rich of contents (Fadhli et al., 2023). The imbalance between its high utilization potential and the limited cultivation development suggests structural challenges in the management of *P. angulata* in Indonesia.

The medicinal potentials of *P. angulata* are derived from the secondary metabolites contained within the plant, such as phenolic content and flavonoid content. Phenolic compound is a large group of secondary metabolites containing one or more aromatic rings with hydroxyl groups (Xu & Wang, 2025). Phenolic compound can be found in many parts of plant with various physiological function, such as antioxidant, protection from ultraviolet radiation, and protection mechanism from pests and diseases (Balasundram et al., 2006). One group derived from phenolic is flavonoid group. Flavonoid is a polyphenol compound contain three aromatic rings as their main structure (Shen et al., 2022). Flavonoid play role in stress reducing mechanism, as a pigment to attract pollinators, and protect plant from pests and diseases (Samanta et al., 2011). Both phenolic and flavonoid compound have pharmaceutical values as antioxidant, anti-inflamation, anti-aging, metabolism regulation, and prevent cardiovascular diseases (Oluwole et al., 2022).

Obtaining a raw material for pharmaceutical products from *P. angulata* is a big challenge in Indonesia due to the lack of conventional cultivation. Moreover, the level of secondary metabolites can be influenced by environmental factors, makes it more difficult to meet the demand (Chaidir et al., 2015). Due to these challenges, one solution to propagate *P. angulata* and obtain its secondary metabolites in a short time, large quantities, and under stable environmental conditions is through *in vitro* callus culture. *In vitro* culture allows us to modify environmental conditions, as well as the quantity and quality of the plant materials to meet industrial demands (Mastuti & Rosyidah, 2021). The purposes of propagating plants through *in vitro* callus are for mass propagation and production of secondary metabolites. This is because callus tissue can be multiplied rapidly and has potential to produces higher levels of secondary metabolites compared to whole plants (Sitorus et al., 2011). Plant tissue culture also allows exposure of controlled stress to enhance the production of secondary metabolites without viability lost.

Phenolic and flavonoid production can be induced under stress condition, such as salinity or salt stress. Salinity stress causes two phases of stresses to plant, osmotic stress and ionic stress (da Silva et al., 2021). Osmotic stress is an inability of plant water intake, causing temporary dehydration; while ionic stress occures when there is excessive ion accumulation of Na^+ and Cl^- inside plant cells causing metabolism abnormalities (Munns & Tester, 2008). Stressed plant will induces reactive oxygen species (ROS) signaling mechanism to activates antioxidant activity, osmoprotectant synthesis, and ion transport protein expression and enhances plant tolerance against salt stress (Ighodaro & Akinloye, 2018). Increased ROS level will activates phenilpropanoid biosynthesis pathway, producing antioxidant compounds such as phenolic and flavonoid to mitigate the oxidative damaging effects (Ahmed et al., 2015). This study is also expected to serve as a reference for industrial-scale herbal medicine manufacturers, enabling them to efficiently obtain high yields of phenolic and flavonoid compounds.

Materials and Methods

Time and location of research. The study was conducted in two stages. The first stage focused on callus induction, aimed at determining the optimal combination of plant growth regulators (PGRs) for callus induction, while the second stage involved the elicitation of callus using NaCl as a salinity stress treatment to identify the optimal concentration for maximizing phenolic and flavonoid accumulation. The first stage was carried out from February to March 2025 at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The second stage conducted from August to December 2025 at the Plant Tissue Culture and Post-Harvest Analysis Laboratory, Faculty of Agriculture, Universitas Padjadjaran.

Tools and materials. The materials used in this experiment included: *P. angulata* seeds collected from Ciparanje Experimental Garden, Universitas Padjadjaran; 1.05% sodium hypochlorite; Murashige and Skoog (MS) medium; 6-benzylaminopurine (6-BAP); 2,4-dichlorophenoxyacetic acid (2,4-D); sodium chloride (NaCl) salt; 80% methanol; distilled water; 10% Folin-Ciocalteu reagent; 7.5% sodium carbonate; 10% aluminum chloride; gallic acid and quercetin standards; 1 M potassium acetate; 2,2-diphenyl-1-picryl-hydrazyl (DPPH); sodium hydroxide; and hydrochloric acid.

The equipment used in this experiment included: a hot plate magnetic stirrer, beakers, an analytical balance, a pH meter, culture bottles, an oven, an autoclave, spatulas, pipettes, micropipettes, forceps, scalpels, Petri dishes, culture scissors, hand sprayers, a spirit lamp, a laminar air flow cabinet (LAFC), culture racks equipped with LED (light-emitting diode) lamps, a thermo-hygrometer, a refrigerator, test tubes, graduated cylinders, volumetric flasks, a mortar and pestle, a shaker, a vortex mixer, a centrifuge, microtubes, cuvettes, and a UV-Vis spectrophotometer.

Experimental Design. First stage of experiment consisted of 12 treatments with 3 replications. Ten culture bottles used for each replication, which containing 3 explants per bottle. The experiment was arranged in a factorial completely randomized design (CRD) with two factors. The first factor was the concentration of the plant growth regulator 6-BAP with three levels of treatments (0, 2, and 4 mg/L), while the second factor was the concentration of the plant growth regulator 2,4-D with four level of treatments (0, 0.5, 1, and 1.5 mg/L).

The optimal medium identified in the first stage of the experiment was utilized as the basal combination for callus induction in the second stage. The second stage consisted of five treatments with four replications. Each replication consisted of 15 culture bottles with three calli per bottle. The experiment was arranged in a single-factor completely randomized design (CRD), with NaCl concentration as the treatment factor at levels of 0, 25, 50, 75, and 100 mM.

First Stage Experiment. *Physalis angulata* L. seeds were obtained from the Ciparanje Experimental Garden, Universitas Padjadjaran. The seeds were harvested from physiologically mature fruits, indicated by their yellow calyx. Seed dormancy was broken using 100 mg/L gibberellin (GA3) solution for 24 hours. The seeds were then sterilized with a 1.05% sodium hypochlorite solution for 15 minutes and rinsed with sterile distilled water three times for 5 minutes each time (Mastuti et al., 2020). The sterilized seeds were then initiated on MS basal medium without any supplementation under aseptic conditions in LAFC. After eight weeks incubation, leaves from the plantlets were used for callus induction. Leaves from the grown plantlets were collected as explants. The criteria of the leaves included located at fourth from both the base and the apex, green in color and not yellowish, and fully opened. The explants were then cut into 1 cm × 1 cm pieces and sub cultured into the treatment medium, with three explants per bottle, under aseptic conditions in the LAFC. The explants were incubated for four weeks in an incubation room at room temperature with 16 hours photoperiod per day.

Second Stage Experiment. Callus from the first experiment were cut into 1 cm × 1 cm x 1 cm pieces and sub cultured into a second stage medium. The basal composition of the medium consisted of MS medium with 1 mg/L BAP and 0.5 mg/L 2,4-D, supplemented with different concentrations of NaCl as experimental treatments. The calluses were then cut into pieces measuring 1 cm × 1 cm. Each bottle consisted of three calluses were incubated for 5 weeks in an incubation room at room temperature with 16 hours photoperiod per day.

Observed Parameters. In the first experiment, leaves explants dedifferentiated into callus. The observed parameters of this stage are the response of callus growth and development to medium with varying concentrations of 6-BAP and 2,4-D. The observed callus growth parameters including the percentage of explants forming callus, callus size, callus fresh weight, callus consistency, and callus color. Callus size was assessed using score diagram on a scale of 1–23 (Figure 1). Callus consistency was evaluated using a consistency scale of 0–5 (Table 1) based on the study by Hidayat et al. (2021). All calli in the first stage experiment were evaluated after four weeks of treatments.

In the second stage of experiment, the observation parameters are including callus secondary metabolites accumulation and antioxidant activity to varying concentrations of NaCl as a salt stress treatment. Callus secondary metabolites accumulation included total phenolic content in gallic acid standard and total flavonoid content in quercetin standard (Yari et al., 2025); while callus antioxidant activity was assessed using IC_{50} DPPH method (Hussen & Endalew, 2023; Shimamura et al., 2014). After five weeks of salinity stress treatment, the callus was dried in an oven at 36°C for 72 hours and subsequently ground into powder. The sample powder was then macerated in 80% methanol for 24 hours while being agitated on a rotational shaker at 100 rpm. Finally, the extract was centrifuged at 4,800 × G for 5 minutes.

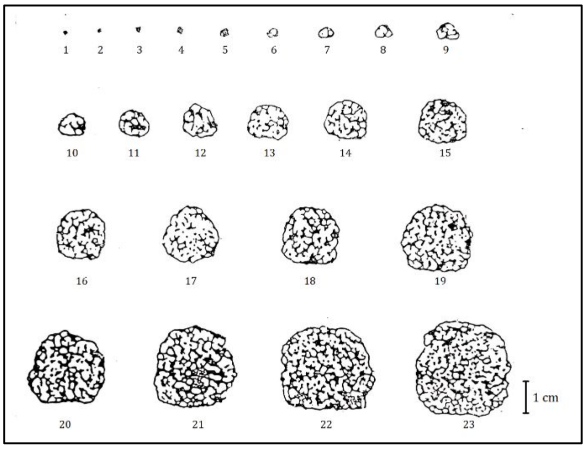


Figure 1. Callus size score

Total Phenolic Content (TPC) was determined by mixing 80 µl of extract or standard solution with 200 µl of 10% Folin-Ciocalteu reagent and homogenized using a vortex for four minutes. Subsequently, 2 mL of 7.5% sodium carbonate was added followed by the addition of distilled water to reach its final volume of 5 mL. The solution was then incubated in the dark room at room temperature for 30 minutes. Absorbance was measured using spectrophotometer at 760 nm wavelength with triplicate analysis. Gallic acid at 25, 50, 100, 200, and 400 mg/L concentration was used as a standard to generate a calibration curve. The TPC values were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

Table 1. Callus consistency score

Score	Consistency	Note
0	Dead and did not form callus	Undifferentiated
1	Friable 1 (cotton-like structure)	Callus with a cotton-like structure
2	Compact callus	Callus surface is glossy and entirely smooth
3	Compact with nodules	Appears on the wounded area of the explant
4	Friable 2 (easily crumbled structure)	Callus with an easily crumbled (friable) structure
5	Friable 2, followed by nodule formation	Callus followed by nodule formation

Total Flavonoid Content (TFC) was determined by mixing 120 µl of extract or standard solution with 1.5 mL of 80% methanol, 100 µl of 10% aluminum chloride, 100 µl of 1 M potassium acetate, and distilled water to reach its final volume of 5 mL. The solution was then incubated in the dark room at room temperature for 40 minutes. The absorbance was measured using spectrophotometer at a wavelength of 415 nm. Quercetin was used as the standard at concentrations of 25, 50, 100, 200, and 400 mg/L to establish a calibration curve. The TFC values were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} DW).

Antioxidant activity was determined using DPPH method. Callus extracts were diluted into 50-2000 mg/L concentrations, and each 2 mL concentration or 2 mL of control was added by 2 mL 0,1 mM DPPH solution. The solutions were then incubated in dark room at room temperature for 30 minutes. The absorbance was measured using spectrophotometer at a wavelength of 517 nm. Inhibitory concentration (IC_{50}) was calculated using direct interpolation method, where antioxidant activities close above and below 50% were selected to determine its regression line and equation according to Li et al. (2015).

$$IC_{50} = x_1 + \frac{50\% - y_1}{y_2 - y_1} \times (x_2 - x_1)$$

ISSN: 1412-4718, eISSN: 2581-138x

Where x_1 and x_2 are the concentrations between 50% inhibitory, while y_1 and y_2 are the inhibitory percentage.

Statistical Analysis. The experimental design used a factorial completely randomized design for the first stage and single completely randomized design for the second stage. Quantitative data were analyzed using analysis of variance (ANOVA) based on the F-test at a 5% significance level. If a significant effect was observed (F calculated > F table), the analysis was followed by Duncan's Multiple Range Test (DMRT)at the 5% level. The analysis was conducted using SPSS software.

Results and Discussion

Percentage of Explants Forming Callus. The first stage of experiment demonstrated interactive effects of the cytokinin 6-BAP and the auxin 2,4-D treatments. Analysis of variance for the percentage of callus formation indicated there is a significant interaction ($p < 0.05$) between both plant growth regulators. The interaction graph of 6-BAP and 2,4-D showed the optimal concentration for each combination after four weeks application (Figure 2). Control treatment induced callus formation percentage only 54%. The application of 6-BAP and 2,4-D to the explants increased the percentage of callus formation compared to the control. The interaction graph of 6-BAP and 2,4-D showed that all concentrations of 6-BAP induced highest callus formation percentages when combined with 2,4-D at 1 mg/L and 1.5 mg/L.

One of the indicators affecting callus induction is the dedifferentiation of explants followed by callus formation. Callus is a mass of unorganized and non-specialized plant cells that has been widely applied in various biotechnology practices such as plant propagation, genetic modification, and secondary metabolite mass production (Thorpe, 2012). Callus *in vitro* culture success rates are influenced by the type of explant, composition of the medium, and plant growth regulators (PGRs). Dedifferentiation and callus formation occurs when explants are treated with appropriate ratios of PGR such as auxin and cytokinin (Su et al., 2011). Cytokinin 6-BAP and auxin 2,4-D are known for their stable structure and have good efficacy for *P. angulata in vitro* culture.

Cytokinin 6-BAP and auxin 2,4-D indicate an interaction in callus formation. These combinations can trigger dedifferentiation and callus formation in plant cells (Su et al., 2011). Explants without 6-BAP and 2,4-D treatment had a potential to fail to form callus or small callus formation. This case happened due to the existence of endogenous hormones from the explant, allowing some cells to divide (Sari et al., 2014). In addition, explant wounding while subculture can be another factor to induce and stimulate cell division and callus formation (Aziz et al., 2014). Wounding in leaf tissue can trigger expression of the cdc2 gene, increasing the competency of cells to undergo cell division (Hemerly et al., 1993).

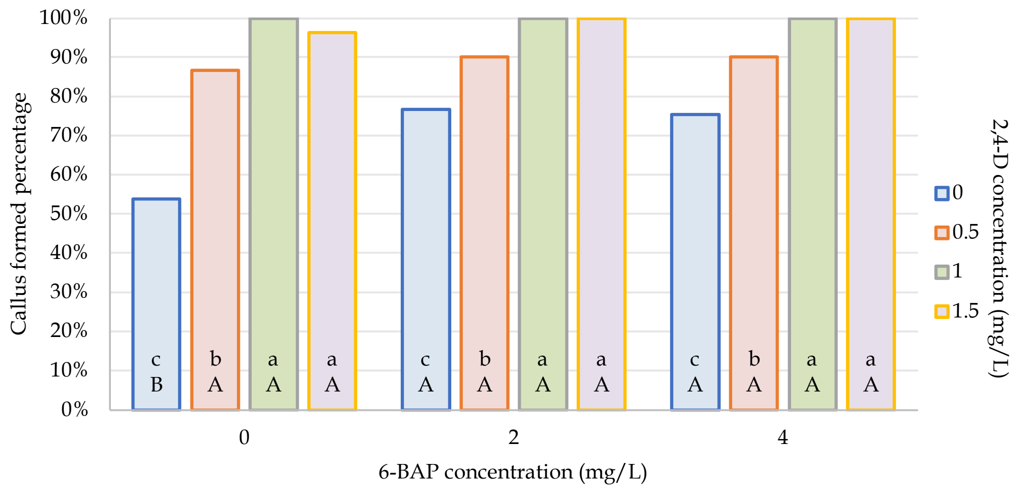


Figure 2. Interaction effect of 6-BAP and 2,4-D on the percentage of callus formation
Note: Means followed by lowercase letters indicate significant differences among 2,4-D concentrations within the same 6-BAP level, while uppercase letters indicate significant differences among 6-BAP concentrations within the same 2,4-D level.

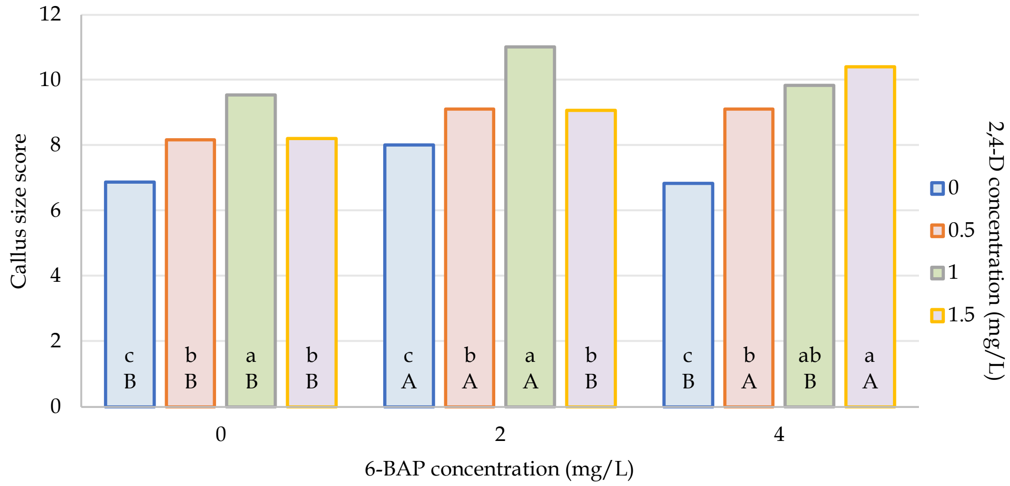


Figure 3. Interaction effect of 6-BAP and 2,4-D on callus size scale

Note: Means followed by lowercase letters indicate significant differences among 2,4-D concentrations within the same 6-BAP level, while uppercase letters indicate significant differences among 6-BAP concentrations within the same 2,4-D level.

Callus Size. There is a significant interaction ($p < 0.05$) between cytokinin 6-BAP and auxin 2,4-D for callus size. This study showed that 6-BAP at 0 and 2 mg/L should be combined with 2,4-D at of 1 mg/L to produce the largest callus size. Cytokinin 6-BAP at 4 mg/L produced the largest callus size when combined with 2,4-D at 1.5 mg/L, although it was not significantly different from the combination with 1 mg/L 2,4-D. High concentrations of 6-BAP combined with 2,4-D at 0 and 1 mg/L tended to reduce callus size (Figure 3). The combination of 6-BAP and 2,4-D increased callus size compared to the control after four weeks (Figure 4).

Culture medium and PGRs composition can greatly affects *P. angulata* callus growth. Callus size is one indicator to assess its suitability to the composition of medium and PGRs. The faster the callus size increased, the faster mass propagation can be done to achieve industrial demands. Tissue swelling is a stage in callus formation that indicates active cell division and elongation (Anggraeni et al., 2022). Proper hormone ratios and tissue woundings in explant trigger continuous cell division and enlarging callus size (Aziz et al., 2014). This condition may involve cdc2 gene expression to prepare cell division and increase tissue growth (Hemerly et al., 1993).

Applying right concentration of PGRs to explants enhances callus size. Tissue swelling and size increase indicate that PGRs combination promotes cell division in callus tissue (Anggraeni et al., 2022). Wounded explant tissue allows easier diffusion of nutrients and PGRs from external medium, which accelerate cell division and tissue swelling (Fitroh et al., 2018). While right concentration of PGRs can increase callus growth, excessive concentrations of PGRs can be toxic, disrupting hormonal balance in the plant tissue, causing stress and inhibiting cell division (Wartina et al., 2011).

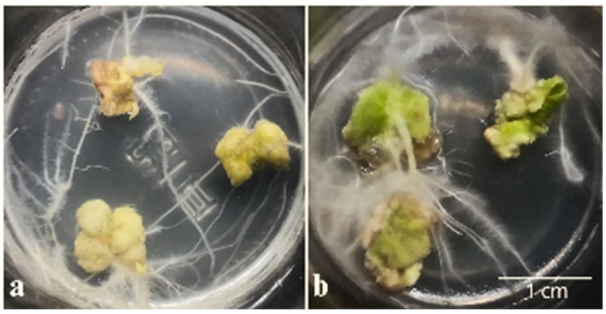


Figure 4. Comparison of callus size on (a) untreated medium and (b) medium with a combination of 2 mg/L of 6-BAP and 1 mg/L of 2,4-D

Callus Fresh Weight. Callus fresh weight was significantly affected ($p < 0.05$) by 6-BAP and 2,4-D interaction. Control treatment produced callus only 83.7 mg of fresh weight at four weeks after subculture. The application of 6-BAP increased callus fresh weight with different

ISSN: 1412-4718, eISSN: 2581-138x

combinations of 2,4-D, although there was no significant difference between 2 mg/L and 4 mg/L of 6-BAP concentrations (Figure 5). The application of 2,4-D increased callus fresh weight until reaching its maximum at 1 mg/L concentration, although there was no significant difference between 0 mg/L of 6-BAP combined with either 1 mg/L or 1.5 mg/L of 2,4-D. At 2 mg/L and 4 mg/L of 6-BAP, the combination with 1.5 mg/L of 2,4-D reduced callus fresh weight and made it not significantly different from 0.5 mg/L of 2,4-D.

Callus growth can be measured through the increase in callus fresh weight. An increase in callus fresh weight indicates optimal cell division (Anggraeni et al., 2022). Application of cytokinins such as 6-BAP and auxins like 2,4-D at appropriate concentration and ratio can be capable to increase callus fresh weights (Fitroh et al., 2018). A synergistic interaction between auxins and cytokinins can stimulate explant's cell division and dedifferentiation, increasing their fresh weight (S. M. Li et al., 2021). High callus fresh weight comes from rapid and active cell division, callus morphology, and high water content (Hidayat et al., 2021). However, excessive concentration of auxin such as 2,4-D can accelerate cell aging, while excessive concentration of cytokinin such as 6-BAP is potentially trigger programmed cell death, hindering cell division and growth (Carimi et al., 2003; Peterson et al., 2016). Callus browning can also inhibit cell division, slowing down the increase in fresh weight (Rybin et al., 2024). On the other hand, too low concentration or inapropriate ratio of PGRs may fail to induce and stimulate cell division (Yücesan et al., 2015)

Callus Consistency and Color. Callus visual observation by its consistency and color can indicate the response of callus development. In general, callus consistencies are categorized into two types: compact and friable. However, Hidayat et al. (2021) utilize a scoring system that classifies callus consistency into six classes for more specific details. This study showed that most calluses had a consistency score 2, which indicate compact callus texture (Table 2). Compact callus usually formed when explants are newly dedifferentiated and the cell structures remain relatively dense and compact. Callus consistency can change over time after being sub cultured onto new medium.

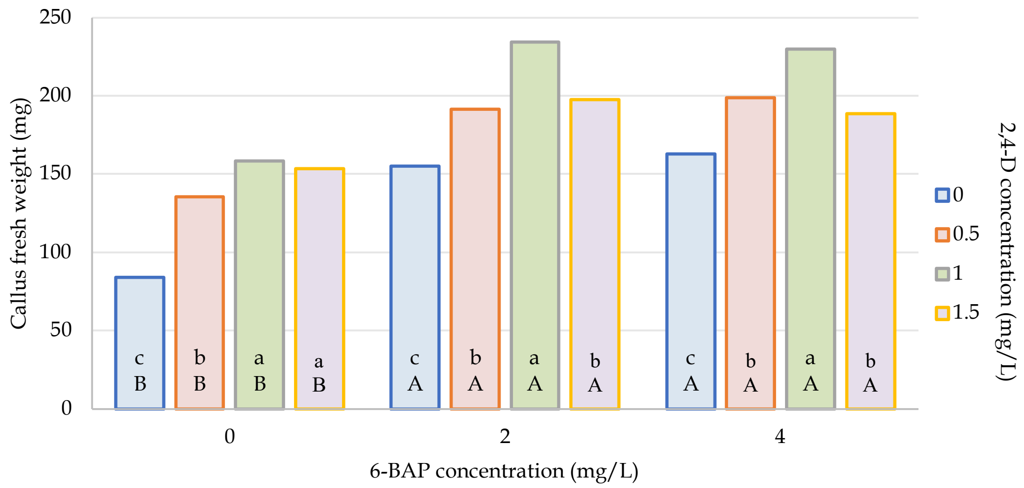


Figure 5. Interaction effect of 6-BAP and 2,4-D on callus fresh weight

Note: Means followed by lowercase letters indicate significant differences among 2,4-D concentrations within the same 6-BAP level, while uppercase letters indicate significant differences among 6-BAP concentrations within the same 2,4-D level

Table 2. Effect of 6-BAP and 2,4-D combinations on callus consistency

2,4-D Concentration	6-BAP Concentration		
	0 mg/L	2 mg/L	4 mg/L
0 mg/L	2	2	2
0.5 mg/L	2	2	2
1 mg/L	2	2	2
1.5 mg/L	2	2	2

Another callus development indicator that can be visually observed is callus color. This study showed that most treatments produced callus with color codes N144A, 151A, and N144C of Royal Horticultural Society Color Chart (The Royal Horticultural Society, London, 2007), which were categorized as yellow-green group (Table 3). Callus from control treatment, the combination of 0 mg/L 6-BAP + 1.5 mg/L 2,4-D, and 2 mg/L 6-BAP + 0.5 mg/L 2,4-D had the color code 151A, which more yellowish compared to other treatments. Treatments that maintained a greener callus color included the combinations of 2 mg/L 6-BAP + 1 mg/L 2,4-D and 2 mg/L 6-BAP + 1.5 mg/L 2,4-D, with color code N144C. This color can indicate that they were able to delay callus browning.

Callus consistency and color can describe its physiological response. Friable callus has loose arranged cells and can be easily broken apart, while compact callus has dense and tightly arranged cells with thick polysaccharide walls which making it harder to separate (Hidayat et al., 2021). Friable callus is suitable for propagation because it can be easily divided and scaled up via suspension cultures (Rasud & Bustaman, 2020), while compact callus is harder to propagate but is more efficient for secondary metabolites accumulation (Indah & Ermavitalini, 2013) due to its structured cell arrangement that potentially facilitates metabolite transport.

Callus color is an indicator that can reflects cell activity level (Rasud & Bustaman, 2020). White to yellowish callus indicates embryogenic and active dividing cells; green callus contains chlorophyll, while brown callus indicates phenolic oxidation or tissue aging (Anggraeni et al., 2022). Callus color may vary depending on the explant used (Hidayat et al., 2021). Low levels of 6-BAP and 2,4-D may trigger phenolic biosynthesis without inducing cell division, leading to phenolic accumulation without increasing its growth. In contrast, high concentrations of 6-BAP may induce programmed cell death, while 2,4-D can increase ethylene levels, accelerating cell senescence (Rybin et al., 2024).

Total Phenolic Content (TPC) Under Salinity Stress

Total phenolic content (TPC) was evaluated using gallic acid standard during second stage of this experiment. *P. angulata* callus showed an increasing trend of TPC although there is a slight decrease compared to control observed at the 25 mM treatment and gradual increase reaching the highest content at the 100 mM NaCl treatment (Table 4). This pattern indicates there are secondary biosynthesis activation of secondary metabolites as a response during salinity stress. Phenolic compounds are identified with its role as a non-enzymatic antioxidant to neutralize ROS accumulation inside plant cells during osmotic and ionic stress caused by salinity. It also can be implied that NaCl treatments at 0-100 mM was still within the callus tolerance threshold to perceive salinity stress.

Phenolic content biosynthesis under salinity stress is related to phenylpropanoid pathway. Salinity stress was divided into two phases, osmotic stress and ionic stress (da Silva et al., 2021). Excessive Na^+ accumulation inside cell during ionic stress will also increase ROS accumulation, which can damage cell by its oxidative reaction (Pungin et al., 2023). Intracellular Ca^{2+} level increased during stress and acting as second messenger to activate calcium sensors such as calcium-dependent protein kinase (CDPK), leading to activation of ROS signaling and transcription factors of defense-related genes and enzymes (Qari & Tarbiyyah, 2021). One of the key regulatory enzymes in phenolic production is phenylalanine ammonia-lyase (PAL) which catalyze phenilalanine, precursor of phenolic compounds (Kumari et al., 2023). Salinity stress signaling pathways allows for redirection of carbon and energy resources from primary metabolism to secondary metabolism for plants defense strategies (Ortiz & Sansinenea, 2023). The increased TPC in plant under salinity stress indicates that phenolic compounds play their role as ROS scavengers to maintain redox homeostasis (Gozdur et al., 2024).

The adaptive response is relevant in this experiment, where external stress factors influence cellular metabolism to reduce oxidative damages caused by stress and consistent with findings reported in another species. A study on *Rumex thyrsiflorus* exposed to salinity for four weeks showed an increase of accumulation of phenolic compound such as gallic acid at 43 mM NaCl concentration by 98.83% on male callus and 129 mM NaCl concentration by 185% on female callus (Gozdur et al., 2024). Another study also revealed that NaCl treatments at 84 and 250 mM for four weeks were able to increase total phenolic content of various *Nigella sative* genotypes (Golkar et al., 2020). These abilities of

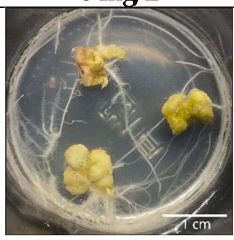
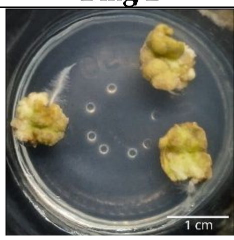
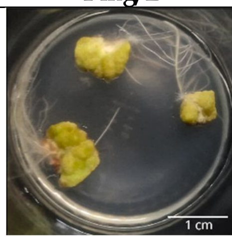
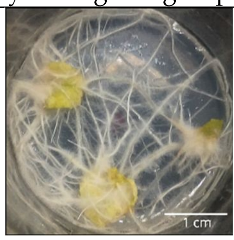
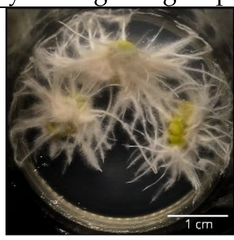
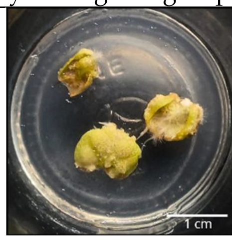
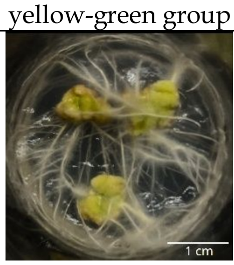
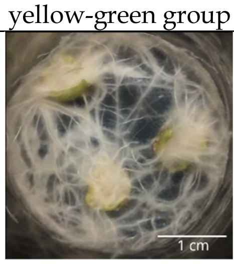
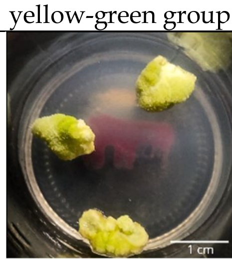
Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

callus to enhance phenolic level under salinity stress indicates a strategic approach to stimulate phenolic accumulation for both physiological and biotechnological application. However, excessive NaCl concentration should be avoided to prevent over-toxicity and physiological damage, which directing into cell death (Bozaba & Kuru, 2024).

Total Flavonoid Content (TFC) Under Salinity Stress

Flavonoid content in *P. angulata* callus was evaluated using quercetin standard during second stage of this experiment. Callus TFC showed increase from control and reaching its highest accumulation at 100 mM NaCl treatment (Table 4). This pattern of value indicates that flavonoid biosynthesis in *P. angulata* callus is responsive to salinity stress treatment. Flavonoid compounds are one part of phenolic compounds that also play role at antioxidant activity to reduce oxidative damage caused by stress. Same with phenolic content, NaCl treatment at 0-100 mM also suggest that it was still within the callus tolerance threshold and acted as an elicitor to stimulate flavonoid biosynthesis. Flavonoid compounds play many roles in plant such as a defense mechanism against various biotics and abiotic stresses or as a non-enzymatic antioxidant (Panche et al., 2016).

Table 3. Callus colors after four weeks under different 6-BAP and 2,4-D combinations

2,4-D Concentration	6-BAP Concentration		
	0 mg/L	2 mg/L	4 mg/L
0 mg/L	 151A yellow-green group	 N144A yellow-green group	 N144A yellow-green group
0.5 mg/L	 N144A yellow-green group	 151A yellow-green group	 N144A yellow-green group
1 mg/L	 N144A yellow-green group	 N144C yellow-green group	 N144A yellow-green group
1.5 mg/L	 151A yellow-green group	 N144C yellow-green group	 N144A yellow-green group

Flavonoid compounds are a part of polyphenols and their biosynthesis also associated with phenylpropanoid pathway. Salinity-induced treatment is frequently used as a mediation to enhance secondary metabolites such as flavonoid by triggering plants adaptive response through its capability as free radical scavengers (Isah, 2019). During exposed to salinity stress, physiology abnormalities will be recognized and inducing many signaling to increase salt tolerance and activate antioxidant enzyme expression including for flavonoid biosynthesis (Chele et al., 2021). Not only PAL, but there are also chalcone synthase (CHS) and chalcone isomerase (CHI) acting as the key of regulatory enzyme controlling flavonoid compounds formation pathways from phenylpropanoid pathway (Mao et al., 2025). From this stage onward, a various kind of flavonoids are synthesized, tailored to their specific physiological roles and function (Kumari et al., 2023). Therefore, the flavonoid content observed in this study implies an adaptive metabolic response of callus tissue to salinity stress treatments.

P. angulata callus response of flavonoid accumulation to salinity treatment observed is aligned with findings reported in various species. A study on *Stevia rebaudiana* callus exposed to salinity for six weeks showed an increase of 40.95% accumulation of flavonoid compound at 100 mM NaCl concentration compared to control (Javed & Gürel, 2019). In *Rumex thyrsiflorus* callus, an increase of TFC, such as quercetin, was observed after four weeks of salinity exposure with 52% increase occurred in male callus at 43 mM NaCl and 221% in female callus at 129 mM NaCl (Gozdur et al., 2024). Another study also revealed that *Rhinacanthus nasutus* callus exposed to salinity stress at 100 mM NaCl concentration for seven days with 14 days of tissue recovery after exposure increased accumulation of total flavonoid content by 28.90% compared to control (Yaowachai et al., 2026). These studies support the observation that salinity stress can act as an effective elicitor to enhance flavonoid accumulation in callus cultures depending on species, NaCl concentration, and exposure duration, including *P. angluata* callus culture.

Antioxidant Activity Under Salinity Stress. Abiotic stress treatment in *P. angulata* callus is intended not only to observe its secondary metabolites content, but also to evaluate its antioxidant capacity. In this second stage of experiment, antioxidant activity was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. Lower IC_{50} value of DPPH reflects a higher ability of the extract to scavenge and neutralize DPPH free radical molecules. The result of this experiment showing gradual decrease of IC_{50} concentration indicates that salinity stress treatment can stimulate the production of antioxidant compounds and reach its higher antioxidant activity at 100 mM NaCl concentration (Table 4). The improvement of antioxidant activity under salinity stress can be associated with secondary metabolites accumulation with radical scavenging abilities.

Salinity stress induces oxidative stress through reactive oxygen species and disrupts cellular redox homeostasis. During salinity stress, secondary metabolites acting as a second line defense of antioxidant after enzymatic antioxidant by donating electrons and neutralize free radical molecules into their harmless form (Ighodaro & Akinloye, 2018). Secondary metabolites such as phenolics and flavonoids antioxidant capacity potential are influenced by its hydroxyl group structures (Gulcin, 2025). Therefore, phenolic and flavonoid content in this research also play role as radical scavenger. Antioxidant activity evaluation method in this experiment used DPPH free radical molecule to induce electron donation from non-enzymatic

Table 4. Comparison of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities after five weeks of callus exposure with different concentrations of NaCl

NaCl Concentration	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Antioxidant Activity IC_{50} (mg/mL)
NaCl 0 mM	3.316	0.433	0.935
NaCl 25 mM	3.309	0.450	0.912
NaCl 50 mM	3.350	0.451	0.905
NaCl 75 mM	3.364	0.469	0.885
NaCl 100 mM	3.494	0.542	0.871

ISSN: 1412-4718, eISSN: 2581-138x

antioxidants, leading to reduction of DPPH molecules into their non-reactive state (Wołosiak et al., 2022). Salinity treatments were able to enhance *P. angulata* callus phenolic and flavonoid accumulation, which also potentially allows antioxidant capacity enhancement.

Several studies have reported antioxidant activity in callus *in vitro* cultures assessed by the DPPH assay, supporting the observation of enhanced radical scavenging capacity under salinity stress. In callus culture of *Thymus daenensis*, 14 days of 100 mM NaCl treatment increased antioxidant activity by 2%, within 0-250 µg/mL observation range of extracts concentration (Golkar et al., 2025). Another research in *Rhinacanthus nasutus* callus showed a decrease of IC_{50} value by 10.16% at 200 mM NaCl concentration exposure for 7 days (Yaowachai et al., 2026). In *Stevia rebaudiana* callus, antioxidant activity was increased by 13.66% at 100 mM NaCl treatment for 45 days (Javed & Gürel, 2019). Based on these studies, salinity stress can be considered as a promising abiotic elicitor to enhance antioxidant activity in *in vitro* callus cultures, including *P. angulata*, through regulation of secondary metabolite production.

Conclusion

Callus induction on *P. angulata in vitro* leaf explants can be triggered by combination of 6-BAP and 2,4-D. The combinations of 6-BAP and 2,4-D are capable to induce dedifferentiation of *P. angulata* leaf explants to form callus. Combination of 6-BAP at 2 mg/L and 2,4-D at 1 mg/L showed the largest callus size, highest fresh weight, and greener callus color compared to other treatments. All treatments produced callus with compact consistencies which are capable for secondary metabolites production. Callus of 6-BAP at 2 mg/L and 2,4-D at 1 mg/L is utilized as secondary metabolites and antioxidant producer by salinity treatments in second stage. Salinity stress treatments can induce *P. angulata* antioxidant levels especially phenolic content, total flavonoid content, and inhibitory concentration at 50% (IC_{50}). NaCl concentration at 100 mM showed the most phenolic and flavonoid accumulation with lowest IC_{50} value, indicates highest antioxidant activity, compared to control. These elevations indicated that salinity stress levels applied in this study did not exceed *P. angulata* callus physiological tolerance.

Acknowledgments

The author expresses gratitude to the Plant Tissue Culture Laboratory and Post-Harvest Analysis Laboratory, Faculty of Agriculture, Universitas Padjadjaran, for the facilities and support provided throughout the course of this research. The author also extends thanks to all laboratory staff and colleagues who assisted in carrying out this study.

References

Ahmed IM, Nadira UA, Bibi N, Cao F, He X, Zhang G, & Wu F. 2015. Secondary metabolism and antioxidants are involved in the tolerance to drought and salinity, separately and combined, in Tibetan wild barley. Environmental and Experimental Botany, 111: 1–12. https://doi.org/10.1016/j.envexpbot.2014.10.003

Anggraeni D, Ismaini L, Surya MI, Rahmi H, & Saputro NW. 2022. Inisiasi kalus daun *Talinum triangulare* (Jacq.) Willd pada beberapa kombinasi konsentrasi zat pengatur tumbuh 2,4-dichlorophenoxyatic acid dan benzyl adenine. Jurnal Agrikultura, 33: 276–288.

Aziz MM, Ratnasari E, & Rahayu YS. 2014. Induksi kalus umbi iles-iles (*Amorphophallus muelleri*) dengan kombinasi konsentrasi 2,4-D dan BAP secara *in vitro*. Lentera Bio, 3: 109–114. http://ejournal.unesa.ac.id/index.php/lenterabio

Balasundram N, Sundram K, & Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry, 99: 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Bozaba TO, & Kuru İS. 2024. The effect of the combined application of elicitors to *Salvia virgata* Jacq. under salinity stress on physiological and antioxidant defense. BMC Plant Biology, 24: 1–12. https://doi.org/10.1186/s12870-024-05443-4

Carimi F, Zottini M, Formentin E, Terzi M, & Lo Schiavo F. 2003. Cytokinins: New apoptotic inducers in plants. Planta, 216: 413–421. https://doi.org/10.1007/s00425-002-0862-x

Chaidir L, Epi, & Taofik A. 2015. Eksplorasi, identifikasi, dan perbanyakan tanaman ciplukan (*Physalis angulata* L.) dengan menggunakan metode generatif dan vegetatif. Jurnal Kajian Islam, Sains, Dan Teknologi, 9: 82–103.

Chele KH, Tinte MM, Piater LA, Dubery IA, & Tugizimana F. 2021. Soil salinity, a serious environmental issue and plant responses: A metabolomics perspective. Metabolites, 11: 1–19. https://doi.org/10.3390/metabo11110724

da Silva AL, do Nascimento MN, de Jesus Carneiro V, dos Santos Neto F, da Silva Leite R, & Tanan TT. 2021. Physiological and biochemical indicators of *Physalis angulata* L. Plants submitted under salinity. Comunicata Scientiae, 12. https://doi.org/10.14295/cs.v12.3450

da Silva Ramos CA, Soares TL, Barroso NS, & Pelacani CR. 2021. Influence of maturity stage on physical and chemical characteristics of fruit and physiological quality of seeds of *Physalis angulata* L. Scientia Horticulturae, 284. https://doi.org/10.1016/j.scienta.2021.110124

Fadhli H, Ruska SL, Furi M, Suhery, WN, Susanti E, & Nasution MR. 2023. Ciplukan (*Physalis angulata* L.): Review tanaman liar yang berpotensi sebagai tanaman obat. Jurnal Farmasi Indonesia, 15: 134–141. https://doi.org/10.35617/jfionline.v15i2.144

Fitroh AI, Dwiyani R, Wijaya IKA, & Yuswanti H. 2018. Pengaruh 2,4-D terhadap induksi kalus daun stroberi (*Fragaria* sp.) dengan media alternatif nutrisi hidroponik AB mix. Agroekoteknologi Tropika, 7: 304–315. https://ojs.unud.ac.id/index.php/JAT304

Golkar P, Alimoradi F, Akbari R, Bazarganipour M, & Peralta-Videa JR. 2025. Exploring the stimulatory effects of graphene oxide-coated zinc oxide nanoparticles on *Thymus daenensis* Celak. callus under salinity stress: insights into biochemical and morphological changes. Plant Cell, Tissue and Organ Culture, 163. https://doi.org/10.1007/s11240-025-03157-5

Golkar P, Bakhshi G, & Vahabi MR. 2020. Phytochemical, biochemical, and growth changes in response to salinity in callus cultures of *Nigella sativa* L. In vitro Cellular and Developmental Biology-Plant, 56: 247–258. https://doi.org/10.1007/s11627-020-10058-z

Gozdur K, Szopa A, & Ślesak H. 2024. Effect of salt stress on growth and phenolic compounds production in callus suspension culture of the dioecious species thyrse sorrel (*Rumex thyrsiflorus* Fingerh.). Plant Cell, Tissue and Organ Culture, 158. https://doi.org/10.1007/s11240-024-02822-5

Gulcin İ. 2025. Antioxidants: a comprehensive review. Archives of Toxicology, 99, 1893–1997. https://doi.org/10.1007/s00204-025-03997-2

Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, & Inzé D. 1993. cdc2a expression in Arabidopsis is linked with competence for cell division. The Plant Cell, 5: 1711–1723. https://doi.org/10.1105/tpc.5.12.1711

Hidayat R, Abdullah, & Aminah. 2021. Pemantapan masa kalus kedelai secara *in vitro* dalam media MS (Murashige dan Skoog) B5 (Gamborg). Jurnal Agrotekmas, 2: 60–70.

Hussen EM, & Endalew SA. (2023). *In vitro* antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. BMC Complementary Medicine and Therapies, 23. https://doi.org/10.1186/s12906-023-03923-y

Ighodaro OM, & Akinloye OA. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54: 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

Indah PN, & Ermavitalini D. 2013. Induksi kalus daun nyamplung (*Calophyllum inophyllum* Linn.) pada beberapa kombinasi konsentrasi 6-benzylaminopurine (BAP) dan 2,4-dichlorophenoxyacetic acid (2,4-D). Jurnal Sains Dan Seni Pomits, 2: 1–6.

Isah T. 2019. Stress and defense responses in plant secondary metabolites production. Biological Research, 52. https://doi.org/10.1186/s40659-019-0246-3

Javed R, & Gürel E. 2019. Salt stress by NaCl alters the physiology and biochemistry of tissue culture-grown *Stevia rebaudiana* Bertoni. Turkish Journal of Agriculture and Forestry, 43: 11–20. https://doi.org/10.3906/tar-1711-71

Kumari A, Kumar V, Ovadia R, & Oren-Shamir M. 2023. Phenylalanine in motion: A tale of an essential molecule with many faces. Biotechnology Advances, 68. https://doi.org/10.1016/j.biotechadv.2023.108246

Li JL, Liu XY, Xie JT, Di YL, Zhu FX. 2015. A comparison of different estimation methods for fungicide EC50 and EC95 values. Journal of Phytopathology, 163: 239-244. https://doi.org/10.1111/jph.12312

Li SM, Zheng HX, Zhang XS, & Sui N. 2021. Cytokinins as central regulators during plant growth and stress response. Plant Cell Reports, 40: 271–282. https://doi.org/10.1007/s00299-020-02612-1

Mao Y, Luo J, & Cai Z. 2025. Biosynthesis and regulatory mechanisms of plant flavonoids: A review. Plants, 14: 1–30. https://doi.org/10.3390/plants14121847

Mastuti R, & Rosyidah M. 2021. Diversity of bioactive secondary metabolites produced by medicinal plants of *Physalis angulata* L. (Ciplukan). IOP Conference Series: Earth and Environmental Science, 743. https://doi.org/10.1088/1755-1315/743/1/012081

Mastuti R, Widoretno W, & Harijati N. 2020. Kultur kalus tanaman obat ciplukan (*Physalis angulata* L.). Biotropika: Journal of Tropical Biology, 8: 26–35.

Munns R, & Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nuranda A, Saleh C, & Yusuf B. 2016. Potensi tumbuhan ciplukan (*Physalis angulata* Linn.) sebagai antioksidan alami. Jurnal Atomik, 1: 5–9.

Oluwole O, Fernando WB, Lumanlan J, Ademuyiwa O, & Jayasena V. 2022. Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health – a review. International Journal of Food Science and Technology, 57: 6326–6335. https://doi.org/10.1111/ijfs.15936

Ortiz A, & Sansinenea E. 2023. Phenylpropanoid derivatives and their role in plants' health and as antimicrobials. Current Microbiology, 80: 1–13. https://doi.org/10.1007/s00284-023-03502-x

Panche AN, Diwan AD, & Chandra SR. 2016. Flavonoids: An overview. Journal of Nutritional Science, 5. https://doi.org/10.1017/jns.2016.41

Peterson MA, McMaster SA, Riechers DE, Skelton J, & Stahlman PW. 2016. 2,4-D past, present, and future: A review. Weed Technology, 30: 303–345. https://doi.org/10.1614/wt-d-15-00131.1

Pungin A, Lartseva L, Loskutnikova V, Shakhov V, Popova E, Skrypnik L, & Krol O. 2023. Effect of salinity stress on phenolic compounds and antioxidant activity in halophytes *Spergularia marina* (L.) Griseb. and *Glaux maritima* L. cultured *in vitro*. Plants, 12: 1–20. https://doi.org/10.3390/plants12091905

Qari SH, & Tarbiyyah I. 2021. The genetic regulation of secondary metabolic pathways in response to salinity and drought as abiotic stresses. Applied Sciences (Switzerland), 11: 1–18. https://doi.org/10.3390/app11156668

Rasud Y, & Bustaman. 2020. Induksi kalus secara *in vitro* dari daun cengkeh (*Syizigium aromaticum* L.) dalam media dengan berbagai konsentrasi auksin. Jurnal Ilmu Pertanian Indonesia, 25: 67–72. https://doi.org/10.18343/jipi.25.1.67

Rybin DA, Sukhova AA, Syomin AA, Zdobnova TA, Berezina EV, & Brilkina AA. 2024. Characteristics of callus and cell suspension cultures of highbush blueberry (*Vaccinium corymbosum* L.) cultivated in the presence of different concentrations of 2,4-D and BAP in a nutrient medium. Plants, 13: 1–18. https://doi.org/10.3390/plants13233279

Samanta A, Das G, & Das SK. 2011. Roles of flavonoids in plants. International Journal of Pharmaceutical Science and Technology, 6: 12-35.

Sari DA, Slameto, & Restanto DP. 2014. Induksi tunas kentang (*Solanum tuberosum* L.) menggunakan BAP (benzyl amino purine). Berkala Ilmiah Pertanian, 1: 1–4.

Shen N, Wang T, Gan Q, Liu S, Wang L, & Jin B. 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chemistry, 383. https://doi.org/10.1016/j.foodchem.2022.132531

Shimamura T, Sumikura Y, Yamazaki T, Tada A, Kashiwagi T, Ishikawa H, Matsui, T., Sugimoto N, Akiyama H, & Ukeda H. 2014. Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives – Inter-laboratory evaluation study –. Analytical Science, 30: 717–721.

Sitorus EN, Hastuti ED, & Setiari N. 2011. Induksi kalus binahong (*Basella rubra* L.) secara *in vitro* pada media Murashige & Skoog dengan konsentrasi sukrosa yang berbeda. Berkala Ilmiah Biologi, 13.

Su Y, Liu Y, & Zhang X. 2011. Auxin-cytokinin interaction regulates meristem development. Molecular Plant, 4: 616–625. https://doi.org/10.1093/mp/ssr007

Thorpe T. 2012. History of plant tissue culture. Methods in Molecular Biology, 877: 9–27. https://doi.org/10.1007/978-1-61779-818-4_2

Vargas-Ponce O, Martínez JS, del Pilar Zamora Tavares M, & Mares LEV. 2016. Traditional management of a small-scale crop of *Physalis angulata* in Western Mexico. Genetic Resources and Crop Evolution, 63: 1383–1395. https://doi.org/10.1007/s10722-015-0326-3

Wartina R, Warnita, & Frizia F. 2011. Pengaruh NAA dan BAP terhadap regenerasi kalus kentang (*Solanum tuberosum* L.) hasil induksi mutasi ethyl methane sulphonate (EMS). Jurnal Tanaman Hortikultura, 2: 1–9.

Wołosiak R, Drużyńska B, Derewiaka D, Piecyk M, Majewska E, Ciecierska M, Worobiej E, & Pakosz P. 2022. Verification of the conditions for determination of antioxidant activity by ABTS and DPPH assays — A practical approach. Molecules, 27. https://doi.org/10.3390/molecules27010050

Xu L & Wang X. 2025. A Comprehensive Review of Phenolic Compounds in Horticultural Plants. International Journal of Molecular Sciences, 26: 1–25. https://doi.org/10.3390/ijms26125767

Yaowachai W, Luecha P, & Taratima W. 2026. Optimizing salinity and drought stress elicitation to enhance phytochemical accumulation and antioxidant activity in *Rhinacanthus nasutus* (L.) Kurz calli. International Journal of Agriculture and Biosciences, 15: 679–692. https://doi.org/10.47278/journal.ijab/2025.220

Yari P, Alirezalu A, & Khalili S. 2025. A comparative study of chemical composition, phenolic compound profile and antioxidant activity of wild grown, field and greenhouse cultivated Physalis (*P. alkekengi* and *P. peruviana*). Food Production, Processing and Nutrition, 7. https://doi.org/10.1186/s43014-024-00287-9

Yücesan BB, Mohammed A, Arslan M, & Gürel E. 2015. Clonal propagation and synthetic seed production from nodal segments of Cape gooseberry (*Physalis peruviana* L.), a tropical fruit plant. Turkish Journal of Agriculture and Forestry, 39: 797–806. https://doi.org/10.3906/tar-1412-86

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Lazuardi AN · Kurniadie D · Umiyati U · Widianto R

Target-site resistance to glyphosate in *Eleusine indica* biotypes from South Sulawesi and Lampung, Indonesia

Abstract. Glyphosate is a non-selective systemic herbicide that has been widely used to control weeds by local corn farmers in South Sulawesi and Lampung Provinces of Indonesia. However, some corn farmers in these two regions were considered that glyphosate herbicide at the standard field dose no longer effective against goosegrass (*Eleusine indica*). To confirms the level of its resistance to glyphosate, seeds of *E. indica* suspected to be glyphosate-resistant were collected from corn fields in Soppeng Regency-South Sulawesi and South Lampung Regency-Lampung (designated as GR-1 and GR-2 respectively), along with a known susceptible biotype from Sumedang Regency-West Java (GS) as a comparison. By using whole-plant pot assay method in a greenhouse at the Ciparanje. A range of seven glyphosate concentrations was applied to the seedlings at the Experimental Farm, representing 0, 0.25, 0.5, 1, 2, 4, and 8 x the standard field rate. Dose–response experiment showed that GR-1 and GR-2 biotypes has a low levels resistance to glyphosate (2.57 and 2.96 folds of each) compared to susceptible biotype (GS). Sequencing results confirmed the Pro-106-Ser mutation in the *EPSPS* genes of the resistant biotypes. This specific amino acid substitution is among the most frequently documented mechanisms of resistance in weed species. Therefore, the shared mutation in the GR-1 and GR-2 biotypes likely confers this resistance. These findings serve as an early warning for corn farmers to manage and prevent further spread of *E. indica* glyphosate-resistant biotypes.

Keywords: Amino acid substitution · *EPSPS* gene · Goosegrass · Herbicide resistance

Submitted: 30 January 2026, Accepted: 10 February 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69589

Lazuardi AN[1*] · Kurniadie D[1] · Umiyati U[1] · Widianto R[2]
[1] Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia
[2] Department of Agrotechnology, Faculty of Science and Technology, Universitas Islam Negeri Sunan Gunung Djati. Jl. A.H. Nasution No. 105, Cipadung, Cibiru, Bandung 40614, West Java, Indonesia.
*Correspondence: ahmad15006@mail.unpad.ac.id

Lazuardi AN, Kurniadie D, Umiyati U, Widianto R. 2026. Target-site resistance to glyphosate in *Eleusine indica* biotypes from South Sulawesi and Lampung, Indonesia. Jurnal Kultivasi, 25 (1): 68-75.

Introduction

Regardless of the cropping system, weeds remain a major factor limiting yields. This has led to a progressive increase in herbicide application frequency within these agricultural systems (Casimero et al., 2022). Of all the herbicides, glyphosate with the IUPAC name N-(phosphonomethyl) glycine has become the most used herbicide in the world, because it has high efficacy against a broad spectrum of weeds (Duke, 2017). In 2014, glyphosate application reached volumes capable of covering 22–30% of global cultivated land, establishing it as the most extensively used pesticide in history (Benbrook, 2016). In the Indonesian agricultural sector, glyphosate constituted 73% of the total herbicide active ingredients utilized. Notably, 7% of this volume was applied during land preparation for corn cultivation (Brookes, 2019).

Goosegrass, scientifically named *Eleusine indica* (L.) Gaertn commonly known as a weed species that have strong competitive ability and seed dispersal potential (Alcántara-de la Cruz et al., 2025; Souza et al., 2024; Takano et al., 2016). Distinguished by C4 metabolism and exceptional adaptability, this species grows rapidly to a height of 30–50 cm. It completes its life cycle within approximately 120 days, during which a single plant can produce a staggering 120,000 seeds (Takano et al., 2016). The presence of *E. indica*, which also known as white crabgrass, silver crabgrass, goosegrass, wiregrass or bullgrass is recognized as a problematic weed species, capable of causing substantial yield losses of 25–90% across both annual and perennial cropping systems (Singh et al., 2025). For instance, in Brazil, corn yield reductions of 18% have been observed with goosegrass densities as low as two plants m^2, escalating to 39% losses at an infestation level of 16 plants/m^2 (Alcántara-de la Cruz et al., 2025). The management of *E. indica* infestation is further complicated by its ability to develop resistance to commonly used herbicides, as the plant is capable of rapidly dominating large areas (Souza et al., 2024).

The first case of glyphosate-resistant population of *E. indica* was detected on a four-year-old orchard in Malaysia, while glyphosate treatments from 720 to 5760 g a.i. ha^{-1} failed to control it (Lee & Ngim, 2000). Since then, glyphosate-resistant biotypes of *E. indica* have been reported in other countries, such as Philippines (Kaundun et al., 2008), USA (Mueller et al., 2011), Brazil (Takano et al., 2016), China (Chen et al., 2023a), and Indonesia (Kurniadie et al., 2023).

E. indica was one of many weeds which commonly found at corn fields in Soppeng Regency-South Sulawesi and South Lampung Regency-Lampung. Corn farmers in these two regions only used glyphosate for more than 10 years and commonly applied 2 to 3 times per year to stop or reduce growth of weeds, especially noxious weeds, before sowing the seeds of corn. However, currently the local corn farmers are being considered that the standard field dose of glyphosate no longer effective against *E. indica*. To determine the level and potential resistance mechanisms, seed samples were collected from two suspected resistant biotypes originating from these regions, along with a known susceptible biotype from Sumedang Regency-West Java as a control. The three biotypes were maintained within a greenhouse environment at the Ciparanje Experimental Farm, Faculty of Agriculture, Universitas Padjadjaran, in which their responses to various glyphosate doses were evaluated. Considering that corn in Indonesia is one of the second strategic food commodities after rice (Muliany & Amzani, 2024), the results of this study can be used as an early warning to mitigate the risk of the presence of resistant weeds in staple crop areas.

Materials and Method

Plant Growing Conditions and Seed Collection. Samples of *E. indica* seeds with suspected resistance to glyphosate were sourced from two independent corn-producing areas; each located in Soppeng Regency (4°19′32.2″ S 120°02′47.0″ E) - South Sulawesi, and South Lampung Regency (5°32′36.8″ S 105°26′28.9 E) - Lampung. These two *E. indica* biotypes (designated as GR-1 and GR-2) were considered by local corn growers as weeds that failed to be controlled by glyphosate. In addition, seeds of a wild *E. indica* at a noncropping area in Sumedang Regency (6°56′4,7″ S 107°47′20,5″ E) – West Java that previously known to be glyphosate-susceptible biotype (GS) were used for comparison in this study (Kurniadie et al., 2023).

The seeds for each *E. indica* biotype population were harvested and collected from 30 mature plants which have fully developed panicles, as the spikelet at the tip have dried out, but the bottom is still slightly green. Seeds underwent cleaning and a one-week sun-drying process to reduce moisture content and promote after-ripening. Samples were then stored in individual paper bags, labeled with their respective GPS coordinates, pending analysis. In mid-August 2025, located in a greenhouse at the Ciparanje, Faculty of Agriculture, Padjadjaran University, these seeds were sown into 25 cm diameter pots filled with a topsoil mix (± 30 seeds per pot of each biotype). To ensure that only seeds of *E. indica* were allowed to sprout and thrive on the soil surface in each pot, previously the potting soil had been sterilized on the autoclave at temperature 120^{o}C and maintain pressure 15 psi for 2 h. During seed germination until it had grown into a *E. indica* plant that is ready to be treated with the glyphosate herbicide, a watering pot was used for watered the plants regularly to maintained optimal soil moisture levels in each pot. Thinning out the *E. indica* plants in each pot were carried out for 21 days to leave 10 individual plant samples per pot, which constituted as an experimental unit.

Glyphosate Dose–Response Experiment. A glyphosate dose–response study was conducted using the whole-plant pot bioassay method (Burgos, 2015). This experiment was conducted as a split-plot design and had two factors, namely the glyphosate dose level and the type of *E. indica* biotype, with three replicates per treatment. Glyphosate (Roundup 486 SL, PT Monagro Kimia, Indonesia) as the whole-plots factor consisted of seven dosage levels: 0, 0.25, 0.5, 1, 2, 4, and 8× of 750 g a.i. ha^{-1} (standard field rate). The second factor was three *E. indica* biotypes (GR-1, GR-2 and GS) as subplots within the application of whole-plots factor. Therefore, distribution of 63 plots (each plot consists of ten *E. indica* plant samples) were prepared to be treated and observed.

Four weeks after sowing, glyphosate herbicide was applied according to the designed dosage for each experimental units of *E. indica plants* in the 4th – 6th leaf growth stage. Foliar treatments with these different doses of glyphosate were applied using a backpack sprayer (semi-automatic) through a single flat-fan nozzles, 400 L ha^{-1} spray volume at a pressure of 138 kPa. The next day after treatments, watering all the plants started again to maintain adequate growth until being harvested to obtained its dry weight.

The magnitude of *E. indica* above ground biomass (AGB) reduction in resistant biotypes (GR-1 and GR-2) and susceptible biotype (GS) due to the impact of each dose of glyphosate (g a.i. ha^{-1}) treatments are recorded at 28 days after treatment (DAT), and compared to untreated controls. The above ground biomass reduction assessment more appropriate to be used rather than total biomass reduction (AGB + below-ground biomass) for several practical and methodological reasons, such as a standardized metric allowing comparison across sites, species, and experiments because root biomass is highly variable to directly reflects the effect of each dose treatments. The above-ground biomass of *E. indica*' survivors in each experimental plot was cut at the base of the stem (at the soil surface), and then the dry weight was obtained by drying the living material in an oven at 80 °C for 48 h before weighing (Ma et al., 2019). The above-ground dry weight biomass reduction in each treated biotype (T) was calculated as the percentage of its growth reduction over dry weight in the untreated control (C) as shown in Equation (1):

$$\text{Biomass Reduction (\%)} = \frac{C-T}{C} \text{ x } 100\%$$

Statistical Analysis. The percentage of dry weight biomass reduction in both resistant (GR-1, GR-2) and susceptible (GS) biotypes was analyzed using ANOVA. The F-test was employed to identify significant differences between means at a significance level of 5%. For the variances that present significance, Duncan's Multiple Range Test (DMRT) at the 95% confidence level was applied, using SmartstatXL software version 3.6.5.4.

Resistance levels were determined by comparing the GR_{50} values; the doses resulting in a 50% reduction in dry weight compared to untreated plants of each biotype. The degree of resistance was expressed as a ratio between the GR_{50} of GR-1 or GR-2 and the susceptible GS biotype. The dose required for 50% growth reduction (GR_{50}) was estimated by fitting the percentage biomass reduction data to a non-linear log-logistic regression model. This analysis produced a sigmoid curve that was fitted to the distribution of growth reduction data (Seefeldt et al., 1995). Data were analyzed using Origin Pro 2018, where the dose–response curve was fitted to a non-linear log-logistic model using the equation:

$$y = C + \frac{D - C}{1 + (x/I_{50})^b}$$

Note: y: dry weight at herbicide dose (x), b=slope, D=upper limit, C=lower limit, I_{50}=the herbicide dose that results reduction of 50% in biomass dry weight.

The resistance index (RI) of a given biotype can then be simply calculated by dividing the GR_{50} of a suspected glyphosate-resistant biotype (GR-1 and GR-2) relatives to the GR_{50} of standard susceptible biotype (GS). The resistance index, based on GR_{50}, is more informative than the actual rate required to kill the resistant population (Heap, 1994). The classification of the level resistance is as follows: R/S > 12: high resistance, R/S = 6–12: moderate resistance, R/S = 2–6: low resistance, R/S < 2: susceptible (Ahmad-Hamdani, 2012).

***EPSPS* Gene Sequence.** Approximately 0.25 g of fresh leaf tissue was collected separately from each sensitive and suspected resistant biotype of *E. indica* when the plants reached 4^{th} – 6^{th} leaf stage. Furthermore, each of these fresh leaf tissues was ground into dry powder using a bead mill, and then vortexed and centrifuged at 2,200 x g for 5 minutes. The pellet obtained at the bottom of the tube was used for DNA extraction (Kaundun et al., 2008). The genomic DNA was extracted using the 'Quick-DNA Magbead Plus kit" (Zymo Research Corp., D4082, Irvine, CA, USA). Quantification of extracted DNA (genomic DNA) was performed with a NanoDrop™ spectrophotometer (Thermo Fisher Scientific). PCR amplification was performed using the "KOD FX Neo kit" (KFX-201Toyobo Co., Ltd., Japan), employed bi-directional sequencing (sequencing DNA fragments in both forward and reverse directions). Primers used to amplify a 330-bp DNA fragment were *EPSPS*-SeqF (5′-CTCTTCTTGGGAATGCTGGA-3′) and *EPSPS*-SeqR (5′-TAACCTTGCCACCAGGTAGCCCTC-3′), which was previously identified as part of the *EPSPS* region.

PCR amplification was performed in a total volume reaction of 50 µL, comprising PCR buffer for KOD FX Neo (25 µL), 10.0 µL of dNTPs (2 mM), 1.5 µL of each primer (0.3 µM), 0.8 µL of KOD FX Neo polymerase, and *E. indica* genomic DNA (1 µL of) (50 ng), with the remaining volume adjusted using 10.2 µL of ddH_2O. PCR condition: 94 °C for 2 min for initial denaturation; 30 cycles of 98 °C denaturation for 10 s and 59 °C annealing for 30 s; and 72 °C final extension for 30 s. The PCR product was then loaded onto a 1% agarose gel with EPSPS _F and EPSPS _R, and sequenced using Sanger sequencing method which was performed by Thermo Fisher Scientific Inc.'s VeritiPro Thermal Cycler, 96 well (Applied Biosystems, Waltham, MA, USA, A48141).

Results and Discussion

Dose–Response Assays. Dose response assays confirmed that the suspected resistant biotypes originated from a corn field in South Sulawesi and Lampung was resistant to glyphosate. The effect of glyphosate dose treatments in Table 1 showed that the suspected resistant biotypes (GR-1 and GR-2) required 2 - 3 folds higher dose (1,500 - 3,000 g a.i ha^{-1}) to achieved an equivalent biomass reduction in susceptible biotype (GS) which was treated with 375 – 750 g a.i ha^{-1}. This indicated that GR-1 and GR-2 biotype have been exhibited a resistance mechanism to the effects glyphosate herbicide in the dose range of 187 g a.i ha^{-1} to 750 g a.i ha^{-1}. Comparable findings have been reported in the first documented case of glyphosate-resistant biotype of *E. indica* in Europe (Lodo et al., 2020).

Table 1. Percentage the biomass reduction of each *E. indica* biotypes

Biotype	Dose of Glyphosate Herbicide (g a.i ha^{-1})						
	0	187	375	750	1500	3000	6000
GS	0.00 a	88.46 b	99.76 c	100.00 c	100.00 c	100.00 c	100.00 c
(West Java)	A	C	C	B	B	A	A
GR-1	0.00 a	16.10 b	66.43 c	72.75 d	95.06 e	100.00 f	100.00 f
(South Sulawesi)	A	B	B	A	A	A	A
GR-2	0.00 a	10.59 b	58.73 c	69.68 d	94.72 e	100.00 f	100.00 f
(Lampung)	A	A	A	A	A	A	A

Note: The average value followed by the same lowercase letter in the row (dose of glyphosate herbicide) and the same uppercase letter in the column (biotype of weed) is not significantly different based on the Duncan test at the 5% level. GS = Glyphosate Susceptible, GR = Glyphosate Resistant

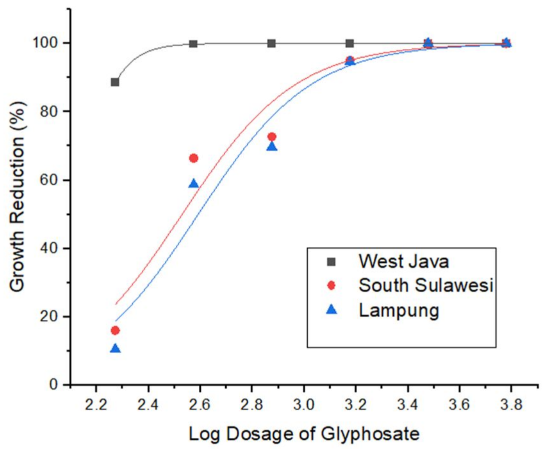


Figure 1. Application of the glyphosate herbicide causes a decrease in growth for both the susceptible (West Java) and resistant biotypes of *E. indica* (South Sulawesi and Lampung)

In the present study, the susceptible of *E. indica* were effectively eliminated at the standard field dose of 360 g a.i ha^{-1}. In contrast, 50% of the resistant biotypes survived even when the glyphosate dose was increased to 1440 g a.i ha^{-1}. In the present study, complete control of GR-1 biotype from South Sulawesi and GR-2 from Lampung was reached at the dose of 3000 g a.i ha^{-1}.

Dose-response curve (Figure 1) revealed a rightward shift in the GR-2 biotype curve compared to the susceptible biotype (GS), indicating greater tolerance to glyphosate. The GR-1 biotype also exhibited a rightward shift, though to a lesser extent, suggesting comparatively lower tolerance. Based on the fitted model, the estimated glyphosate dose required to reduce the biomass dry weight of the GR-1 and GR-2 biotype by 50% (GR_{50} value) was 338 and 390 g a.i ha^{-1} respectively, which was significantly higher than that of the GS biotype (131 g a.i ha^{-1}). On the basis of GR_{50} values (Table 2; Figure 1), the GR-1 and GR-2 biotypes was 2.57 and 2.96-fold respectively to glyphosate; compared to the GS-susceptible biotype. Based on these values, both of GR-1 and GR-2 biotypes demonstrate a decline in sensitivity to glyphosate herbicide, suggesting had a low-level of resistance (R/S value of < 4) (Ahmad-Hamdani et al., 2012). Consequently, glyphosate has become ineffective for managing these biotypes, even though they are classified within the low-resistance category. To ensure effective suppression, alternative strategies must be employed, such as rotating herbicides with different modes of action (MoA) and implementing non-chemical control methods (Ramos et al., 2021; Perotti et al., 2020; PAN, 2023; Nath et al., 2024; HRAC, 2025; Singh et al., 2025; Ravi & Kamal, 2025).

PCR Amplification and Analysis of *EPSPS* Gene. Alignment of *EPSPS* gene sequences identified a point mutation at codon 106, where a CCA-to-TCA transition resulted in a Pro-106-Ser amino acid replacement in the resistant biotypes (Table 3). This mutation confers glyphosate resistance in *E. indica* biotypes from a corn field in South Sulawesi and Lampung Provinces. The Pro-106-Ser mutation was reported as the first glyphosate-resistant in *E. indica* population (Baerson et al., 2002), and has subsequently been documented in resistant populations from Brazil (Takano et al., 2019) and China (Chen et al., 2023a). Mutations at codon 106 of EPSPS are among the most frequently reported target-site resistance mechanisms, alongside those occurring at codon 102 (Gaines et al., 2020; Kurniadie et al., 2023).

Table 2. Resistance index (RI) and GR_{50}

Biotypes	GR_{50}	r^2	RI	Resistance Level *)
GS (West Java)	131.36	1.00	1.00	Susceptible
GR-1 (South Sulawesi)	337.78	0.94	2.57	Low
GR-2 (Lampung)	389.87	0.95	2.96	Low

Note: *) = Ahmad-Hamdani et al., 2012. GS = Glyphosate Susceptible, GR = Glyphosate Resistant

Table 3. Comparison of deduced amino acid sequences of the *EPSPS* gene around position 106 in susceptible and resistant *Eleusine indica* biotypes

Biotype	Position	101	102	103	104	105	106
Susceptible *)	Sequence:	GGA	ACT	GCA	ATG	CGA	CCA
	Amino Acid:	(Gly)	(Thr)	(Ala)	(Met)	(Arg)	(Pro)
South Sulawesi	Sequence:	GGA	ACT	GCA	ATG	CGA	**TCA**
	Amino Acid:	(Gly)	(Thr)	(Ala)	(Met)	(Arg)	**(Ser)**
Lampung	Sequence:	GGA	ACT	GCA	ATG	CGA	**TCA**
	Amino Acid:	(Gly)	(Thr)	(Ala)	(Met)	(Arg)	**(Ser)**

Note: *) = The nucleotide sequence related to the amino acid position of the susceptible *E. indica* biotype is the same as the GenBank Accession reference (AY157642.1) (Takano et al., 2019)

The proline substitution by serine at position 106 in the *EPSPS* gene of resistant biotypes modifies the structural and functional conformation the active site of EPSPS, which subsequently lead to reducing its affinity for glyphosate (Han et al., 2016; Fonseca et al., 2020). This increases the inhibitory constant (Ki)—the concentration of glyphosate required to reduce EPSPS activity by 50%, as more glyphosate is required to inhibit EPSPS mutant activity with its natural substrate (PEP: phosphoenolpyruvate), under normal physiological conditions (Gaines et al., 2020). In addition, the affinity of the EPSPS mutant with Pro-106-Ser substitution tended to bind for PEP more than glyphosate compared with natural EPSPS (Chen et al., 2023b). As a result, this present study found a significant difference in the glyphosate susceptibility between the susceptible and resistant biotypes of *E. indica* occurring in corn fields from South Sulawesi and Lampung.

Conclusion

This study provides compelling evidence of emerging glyphosate resistance in *E. indica* biotypes collected from maize fields in South Sulawesi (GR-1) and Lampung (GR-2). Dose–response assays categorized these biotypes as having low-level resistance, with resistance indices of 2.6 and 3.0, respectively. Sequence analysis of the *EPSPS* gene confirmed the Pro-106-Ser substitution, a characterized target-site resistance (TSR) mutation that diminishes glyphosate's binding affinity to the enzyme's active site.

Although GR-1 and GR-2 biotypes being in low level resistance to glyphosate, the detection of this mutation highlights the potential for rapid escalation if glyphosate continues to be applied as the sole control measure. The findings underscore the importance of several management approach, including to rotate herbicides with different mode of action which is integrated with non-chemical control methods, and establish resistance monitoring programs. Therefore, these needs to be explained to local corn farmers in South Sulawesi and Lampung Provinces, so that they can involve in preventing its spread to other locations.

Acknowledgments

The authors acknowledge the Rector of Universitas Padjadjaran for providing financial support to cover the publication costs of this manuscript

Reference

Ahmad-Hamdani MS, Owen MJ, Yu Q, Powles SB. 2012. ACCase-inhibiting herbicide-resistant *Avena* spp. Populations from the Western Australian Grain Belt. Weed Technology, 26: 130–136.

Alcántara-de la Cruz R, Silva LBXda, Takano HK, Barcellos Júnior LH, Mendes KF. 2025. The rise of *Eleusine indica* as Brazil's most troublesome weed. Agronomy, 15(8): 1759.

Benbrook CM. 2016. Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur, 28: 3.

Brookes G. 2019. Glyphosate use in Asia and implications of possible restrictions on its use. Ag Bio Forum, 22(1): 1-26.

Burgos NR. 2015. Whole-plant and seed bioassays for resistance confirmation. Weed Science, 63(sp1): 152–165.

Casimero M, Abit MJ, Ramirez AH, Dimaano NG, Mendoza J. 2022. Herbicide use history and weed management in Southeast Asia. Adv Weed Sci, 40(Spe1): e020220054.

Chen J, Li Z, Cui H, Yu H, Li X. 2023a. Gene amplification of *EPSPS* with a mutation in conserved region: the evolved glyphosate resistance mechanism in *Eleusine indica.* Agronomy, 13(3): 699.

Chen J, Cui H, Li Z, Yu H, Hou Q, Li X. 2023b. Potential Role of EPSPS Mutations in the Resistance of *Eleusine indica* to Glyphosate. International Journal of Molecular Sciences, 24(9): 8250.

Duke S. 2017. The history and current status of glyphosate. Pest Manag Sci, 74: 1027–1034.

Fonseca ECM, Da Costa KS, Lameira J, Alves CN, Lima AH. 2020. Investigation of the target-site resistance of EPSP synthase mutants P106T and T102I/P106S againts glyphosate. RSC Advances, 10: 44352-44360.

Gaines TA, Duke SO, Morran S, Rigon CAG, Trane PJ, Küpper A, Dayan FE. 2020. Mechanisms of evolved herbicide Resistance. J. Biol. Chem, 295(30): 10307–10330.

Han H, Yu Q, Widderick MJ, Powles SB. 2016. Target-site EPSPS Pro-106 mutations: Sufficient to endow glyphosate resistance in polyploid *Echinochloa colona*?. Pest Mangement Science, 72(2): 264-271.

Heap IM. 1994. Identification and documentation of herbicide resistance. Phytoprotection, 75(4): 85–90.

HRAC (Herbicide Resistance Action Committee). 2025. Guideline to the management of herbicide resistance. Crop Life International, Belgium.

Kaundun SS, Zelaya IA, Dale RP, Lycett AJ, Carter P, Sharples KR, Mcidoe E. 2008. Importance of the Pro-106-Ser target-mutation in conferring resistance glyphosate in a goosegrass (*Eleusine indica*) population from Philippines. Weed Science, 56: 637-646.

Kurniadie D, Widianto R, Umiyati U, Widayat D, Nasahi C, Budiawan A. 2023. Management of *Eleusine indica* (L.) Gaertn resistance to glyphosate herbicide in Indonesia. Agronomy, 13(6): 1649.

Lee LJ and Ngim J. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L.) Gaertn) in Malaysia. Pest Manag Sci, 56:336-339.

Loddo D, Imperatore G, Milani A, Panozzo S, Farinati S, Sattin M, Zanin G. 2020. First report of glyphosate-resistant biotype of *Eleusine indica* (L.) Gaertn. in Europe. Agronomy, 10(11): 1692.

Ma X, Ma Y, Wu H, Ren X, Jiang W, Ma Y. 2019. Emergence timing affects growth and reproduction of goosegrass (*Eleusine indica*). Weed Technology, 33(6): 833-839.

Muliany PH, and Amzani NI. 2024. Outlook Jagung 2024. *Agricultural data and information center*, Ministry of Agriculture, Indonesia.

Mueller TC, Barnett KA, Brosnan JT, Steckel LE. 2011. Glyphosate-resistant goosegrass (*Eleusine indica*) confirmed in Tennessee. Weed Science, 59 (4): 562–566.

Nath CP, Singh RG, Choudhary VK, Datta D, Nandan R, Singh SS. 2024. Challenges and alternatives of herbicide-based weed management. Agronomy, 14(1): 126.

PAN (Pesticide Action Network) Europe. 2023. Weed management: alternatives to the use of glyphosate. Third Edition.

Perotti VE, Larran AS, Palmieri VE, Martinatto AK, Permingeat HR. 2020. Herbicide resistant weeds: a call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. Plant Science, 290: 110255.

Ramos GdaC, Santos PHVdos, Schendenffeldt BF, Hirata ACS, Monquero, P.A. 2021. Management of *Eleusine indica* and *Digitaria insularis* with herbicides in association with cover plants. Journal of Experimental Agriculture International, 43(2): 21-32.

Ravi and Kamal. 2025. Herbicide resistance in weeds: challenges, mechanisms, and integrated approaches: a review. International Journal of Research in Agronomy, 8(1): 393-397.

Seefeldt SS, Jensen JE, Feurst EP. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technology, 9(2): 218–227.

Singh A, Karaca E, Russell D, McElroy S, Maity A. 2025. Biology and status of herbicide resistant goosegrass and its control options in Alabama. Plains Press, Jan. 2025.

Souza MF, Henckes JR, Zobiole LHS, Oliveira RS, Braz GBP, Constantin J, Machado FG, Amarante AA, Ferreira CJB. 2024. Competitive response of maize against glyphosate-resistant *Digitaria insularis* and *Eleusine indica*. Crop Protection, 183: 106760.

Takano HK, Oliveira JR RS, Constantin J, Braz GBP, Padovese JC. 2016. Growth, development, and seed production of goosegrass. Planta Daninha, 34(2): 249-257.

Takano HK, Mendes RR, Scoz LB, Lopez Ovejero RF, Constantin J, Gaines TA, Westra P, Dayan FE, Oliveira RS 2019. Proline-106 EPSPS mutation imparting glyphosate resistance in goosegrass (*Eleusine indica*) emerges in South America. Weed Science, 67 (1): 48–56.

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Tarigan PL · Moeljani IR · Suryandika F · Munarko H

Response of chlorophyll, morphology, and yield of several East Java superior soybean (*Glycine max* L.) varieties to levels of salinity

Abstract. Soybean production has been extended to saline areas. This development is a strategy to expand planting areas to suboptimal land to increase worldwide production. To support cultivation in these locations, it is necessary to develop superior varieties that are resistant to salinity and provide supporting technological inputs. In saline conditions, soybeans may face stress that leads to morphological, physiological, and yield disturbances. Consequently, the response of several soybeans in East Java should be observed. This study is a pot experiment and arranged in a Factorial Completely Randomized Design (CRD) consisting of two factors, soybean varieties and NaCl concentrations. The treatments comprise several NaCl levels: 0 g/L (non-saline), 5 g/L (moderate salinity), 15 g/L (high salinity), and 25 g/L (very high salinity). The observation used several parameters: chlorophyll content, plant height, leaf number, flowering time, pod forming time, number of pods, number of seeds, weight of 100 seeds, and harvest time. The soybean varieties used were Anjasmoro, Wilis, Dering 1, and Dering 3. Anjasmoro was identified as the soybean variety most tolerant to salinity stress based on chlorophyll content, morphology, and yield.

Keywords: Chlorophyll · NaCl · Soybean · Salinity

Submitted: 8 February 2026, Accepted: 29 April 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69758

Tarigan PL[1*] · Moeljani IR[1] · Suryandika F[1] · Munarko H[2]
[1]Program Study of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. Jl. Rungkut Madya, Gn. Anyar, Surabaya 60294, East Java, Indonesia
[2]Program Study of Food Technology, Faculty of Engineering and Science, Universitas Pembangunan Nasional "Veteran" Jawa Timur. Jl. Rungkut Madya, Gn. Anyar, Surabaya 60294, East Java, Indonesia
*Correspondence: puji.lestari.agro@upnjatim.ac.id

Tarigan PL, Moeljani IR, Suryandika F, Munarko H. 2026. Response of chlorophyll, morphology, and yield of several East Java superior soybean (*Glycine max* L.) varieties to levels of salinity. Jurnal Kultivasi, 25 (1): 76-86.

Introduction

Soybean (*Glycine max* L.) faces many problems and limits the potential varieties in yield and quality, affected by the regions (Vogel et al., 2021). Salinity is one of the harmful threats to plant productivity, particularly in high concentrations (Dong et al., 2025). Among the factors, salinity affects several physiological and morphological characteristics in plants, leading to severe yield reductions (Otie et al., 2021). The continuous transformation of arable farming to non-arable land has led sustainable agriculture systems to extend into marginal areas (Sackey et al., 2025). Considering those areas with saline tendencies, successful extensification requires a strict selection and evaluation of genotypes tolerant to these conditions (Jha et al., 2022). Moreover, increasing global warming is increasing sea levels, leading to saltwater introduction to aquifers and agroecosystems (Chen and Mueller, 2018; Sultan et al., 2023).

NaCl on plant tissue can cause disturbance in physiological and molecular activity, such as the regulation process, decreased photosynthesis, and thus an excess of reactive oxygen species (Kumari et al., 2024; Mohajan et al., 2025). This is related to chlorophyll synthesis decreasing due to salinity by activating the chlorophyllase enzyme (Al-Ashkar et al., 2020; Alam et al., 2022). Due to the reduced photosynthetic capacity associated with salt stress, there are many fewer available resources, leading to inhibition of cell growth and expansion (Zelm et al., 2020; Peng et al., 2025). Because of low water potential due to increased salt levels, thus reducing the ability of plant cells to absorb water and reduces cell turgor pressure, which ultimately affects cell expansion. These initiate the shrinkage of plant tissue and the release of water from plant cells (Van Zelm et al., 2020;).

Salinity led to noticeable changes in the plant's shape, such as shorter plant height, fewer leaves on each plant, thinner and shorter stems, and less growth in both the shoots and roots (Kokebie et al., 2024). Salinity stress in soybean cultivation on tidal lands can reduce growth and yield, particularly during the early stages of plant development (Basuni, 2017; Nasution et al., 2024). When plants are exposed to salt for a long time, their leaves start to age, turn yellow, and eventually fall off. Too much salt can lower the amount of pigment, like chlorophyll, in the leaves and also decrease the area where photosynthesis can happen efficiently (Razmjooei et al., 2022; Mousavi et al., 2022).

Plant reaction to salinity is a multifaceted trait formed from the integration of various physiological processes. Thus, the morphological trait is not enough to evaluate when facing the salinity condition. Comprehensive studies should integrate physiological and biochemical parameters to characterize adaptation to salinity (Hasegawa et al., 2000; Bougrine et al., 2025).

Salinity has been identified as an important abiotic stress constraining the spread and productivity of soybean on marginal and salinized soils. Although the influences of salinity stress on soybean growth have been well documented, there is little information available on varietal differences in physiological responses, particularly to the stability of chlorophyll and photosynthetic activity. It is therefore very important to develop such screening techniques as well as experiments aimed at comparing soybean varieties for salt stress tolerance. Hence, the morphological, physiological, and yield characteristics of salt tolerance are the principal stages in establishing resistant soybean varieties.

Materials and Methods

The research was conducted at the Experimental Field, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The activities were carried out from October to November 2023. The materials used are soybean seeds, NaCl, polybag, planting media, Rhizobium, Urea fertilizer, KCl, and TSP. The equipment used are hoe, watering tools, buckets, shade netting, labels, stationery, and a camera.

The study was conducted as pot experiment and arranged in a Factorial Completely Randomized Design (CRD) consisting of soybean varieties and NaCl concentrations. The treatments consisted of several NaCl concentration levels: S0=0 g/L (non saline), S1=5 g/L (moderate salinity), S2=15 g/L (high salinity), and S3=25 g/L (very high salinity). The soybean varieties used were: V1=Anjasmoro, V2=Wilis, V3=Dering 1, and V4=Dering 3. There were 16 treatment combinations, with each treatment replicated three times, resulting in 48 experimental units.

The treatments were applied in accordance with the salinity classification by Rhoades and

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Loveday (1992) and Kristiono et al. (2013). The treatment volume applied was 500 mL per polybag. Treatments were applied at 10, 30, and 50 days after planting (DAP), and fertilization was performed at 7 and 21 DAP. The fertilizer doses per polybag were Urea = 0.4 g/plant, KCl = 0.4 g/plant, and TSP = 0.3 g/plant. Besides that, the application of Rhizobium when planting as a soybean seed cover and additionally, paranet 90% during early growth to avoid high temperatures.

The observation parameters are plant height, leaf number, chlorophyll content (a, b, and total), flowering time, pod forming time, number of pods, number of seeds, weight of 100 seeds, and harvest time. Chlorophyll content was analyzed by the Harbourne (1987) method. The data were analyzed using SAS 9.4 software. The data underwent analysis of variance (ANOVA), followed by further testing using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Results and Discussion

Table 1 shows that there is no interaction between salinities and varieties on plant height. Growth is a key factor in determining plant tolerance to stress. Salinity is a limiting element for plants. Soybeans can grow in a wide range of salinities. The level of soybean resistance is determined genetically by the cultivar individually. In the first week, the Dering 3 was significantly different from Anjasmoro and Dering 1 in plant height. From the second to the fifth week, all kinds except Dering 1 showed more consistent improvements. Among the tested soybean varieties, Dering 3 indicates the highest plant height mean, followed by Anjasmoro and Wilis, while Dering 1 consistently showed the lowest plant height across observation periods. In general, the soybean plant height of the Anjasmoro and Wilis varieties was higher than that of Dering 1 and Dering 3, consistent with the morphological characteristics of these varieties under normal conditions. Based on the mean plant height in this study, Dering 3 is highly tolerant, Anjasmoro and Wilis are moderately tolerant, while Dering 1 is susceptible to salinity. According to Hamayun et al. (2010) and Pujiati et al. (2021), salinity stress reduced gibberellic acid production, which in turn reduced cell expansion, thereby affecting crop growth. Novita et al. (2023) added that the differences in plant height among various species in different environtment condition could be attributed to their genetic ability to adapt to salinity stress conditions.

In the first week, salinity factors were not treated. From the 14 DAP to the 28 DAP, salinity had no significant effect on plant height. A significant effect became evident only in the fifth week, when very high salinity markedly reduced plant height compared with the non-saline treatment. In moderate to very high saline tolerance, the mechanism instinct actived in the way to stay alive. In the early stage, treatment at 10 DAP does not affect plant height compared to non-saline as a control in 14, 21, and 28 DAP. Treatment at 30 DAP, very high saline decrease on plant height.

Table 1. Plant height of superior soybean (*Glycine max* L.) varieties from East Java on several levels of salinity at 7, 14, 21, 28, and 35 DAP

	Plant Height				
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
Varieties	cm	cm	cm	cm	cm
V1 (Anjasmoro)	9.92b	23.1a	33.1a	39.5a	48.6a
V2 (Wilis)	10.58ab	23.7a	32.3a	39.3a	47.0a
V3 (Dering 1)	8.25c	15.0b	21.6b	28.0b	35.2b
V4 (Dering 3)	12.08a	23.1a	38.4a	44.0a	53.0a
Salinities (NaCl)					
S0 (0 g/L) (Non Saline)	8.83b	21.0	29.7	38.9	49.3a
S1 (5 g/L) (Moderate Saline)	9.92ab	21.4	30.9	38.8	49.0ab
S2 (15 g/L) (Very Saline)	10.58a	21.5	31.1	37.5	44.3ab
S3 (25 g/L) (Very High Saline)	11.50a	21.2	30.0	35.7	41.3b

Note: Means followed by the same letter in the table are not significantly different at 5% DMRT Soybean growth salinity. DAP: Days After Planting

The potential of salinity to inhibit plant growth through ion toxicity, nutritional imbalances, or a combination of these factors is linked to a decline in growth indices (Wasif et al., 2023; Kokebie et al., 2024). Sodium chloride (NaCl) is also taken up as a solution through the xylem and translocated to the leaves. Given that the plants are still able to grow, it is suggested that soybean tolerance is achieved through the compartmentalization of excess ions into cell vacuoles. Machado & Serralheiro (2017); Aboh & Eyong (2023) reported that plants grow less in saline environments because of less water availability, greater stomatal resistance, and excessive ion Na and Cl accumulation in the plant tissue.

Salt stress disturbs ionic homeostasis in plants by facilitating excessive Na^+ influx, thereby impeding the absorption of important ions such as K^+, Ca^{2+}, and Mg^{2+}. The negative effect of disturbance cell system tissue, ion exchange, activity adjustment, and finally interrupts physiological and metabolic functions (Wang et al., 2022; Han et al., 2025).

The salt tension that causes ionic and osmotic pressure interferes with normal cell function (Javaid et al., 2024; Tanveer et al., 2025). Hence, high salinity inhibits meristem activity and cell elongation in plants (Dolatabadian et al., 2011; Narayana et al., 2023).

Table 2. Leaf number of superior soybean (*Glycine max* L.) varieties from East Java on several levels of salinity 2, 3, 4, and 5 weeks

Varieties	Leaf Number			
	14 DAP	21 DAP	28 DAP	35 DAP
V1 (Anjasmoro)	1.75a	3.66a	5.08a	7.16a
V2 (Wilis)	1.58a	3.83a	5.75a	7.66a
V3 (Dering 1)	1.08b	2.57b	4.16b	5.41b
V4 (Dering 3)	1.83a	3.08a	5.58a	8.33a
Salinities (NaCl)				
S0 (0 g/L) (Non Saline)	1.58	3.33	5.33	7.58ab
S1 (5 g/L) (Moderate Saline)	1.66	3.91	5.16	8.25a
S2 (15 g/L) (Very Saline)	1.58	3.83	5.25	6.75bc
S3 (25 g/L) (Very High Saline)	1.41	3.25	4.83	6.00c

Note: Means followed by the same letter in the table are not significantly different at 5% DMRT. DAP: Days After Planting

Table 3. Chlorophyll a content of superior soybean (*Glycine max* L.) varieties from East Java on several levels of salinity

Treatment	Chloropyll a
0 g/L (non saline) + Anjasmoro	17,68 ± 0,29
0 g/L (non saline) + Wilis	17,51 ± 0,49
0 g/L (non saline) + Dering 1	16,97 ± 0,06
0 g/L (non saline) + Dering 3	17,03 ± 0,15
5 g/L (moderate salinity) + Anjasmoro	17,04 ± 0,16
5 g/L (moderate salinity) + Wilis	16,92 ± 0,07
5 g/L (moderate salinity) + Dering 1	17,21 ± 0,19
5 g/L (moderate salinity) + Dering 3	17,16 ± 0,21
15 g/L (high salinity) + Anjasmoro	17,08 ± 0,23
15 g/L (high salinity) + Wilis	17,04 ± 0,16
15 g/L (high salinity) + Dering 1	17,29 ± 0,34
15 g/L (high salinity) + Dering 3	17,05 ± 0,18
25 g/L (very high salinity) + Anjasmoro	17,03 ± 0,14
25 g/L (very high salinity) + Wilis	17,11 ± 0,27
25 g/L (very high salinity) + Dering 1	17,30 ± 0,33
25 g/L (very high salinity) + Dering 3	17,04 ± 0,17

Note: Data are presented as mean ± standard deviation

Table 4. Chlorophyll b and Total content of superior soybean (*Glycine max* L.) varieties from East Java on several levels of salinity

Salinities \ Varieties	V1 (Anjasmoro)	V2 (Wilis)	V3 (Dering 1)	V4 (Dering 3)
	Chlorophyll b Content			
S0 (0 g/L) (Non Saline)	19.68f	21.15e	27.08bc	19.13f
S1 (5 g/L) (Moderate Saline)	26.64bc	28.18ab	25.59cd	30.12a
S2 (15 g/L) (Very Saline)	28.08ab	25.94cd	29.87a	26.80bc
S3 (25 g/L) (Very High Saline	26.86bc	25.97cd	23.64d	26.98bc
	Total Chlorophyll Content			
S0 (0 g/L) (Non Saline)	36.99g	38.59f	44.08c	36.56g
S1 (5 g/L) (Moderate Saline)	44.01c	45.04b	43.14d	47.57a
S2 (15 g/L) (Very Saline)	45.79b	43.01d	47.06a	43.94d
S3 (25 g/L) (Very High Saline	43.95c	43.25d	41.10e	44.06c

Note: Means followed by the same letter in the table are not significantly different at 5% DMRT.

Table 2 shows that there is no interaction between salinities and varieties. When plants are exposed to environmental conditions, leaf organs represent the most apparent response. Changes in the leaves of broad-leaved and trifoliate soybean plants are readily observable. Dering 3 consistently exhibited the highest number of leaves at all observation times, although it was not significantly different from Anjasmoro and Wilis. On the other hand, Dering 1 always exhibited the least number of leaves and differed significantly from the rest of the cultivars. In terms of the varietal effect of the number of leaves, Dering 3, Anjasmoro, and Wilis exhibited better adaptability.

The genetic diversity for salt tolerance in soybean is mainly due to the variation in ion exclusion from shoots, particularly photosynthetically active mesophyll cells (Thanh et al., 2023; Açıkbaş et al., 2023). Furthermore, salinity causes certain genes to be expressed, which increases positive regulation of antioxidant enzymes, thereby improving salt plant resilience (Hasanuzzaman et al., 2022).

The effect of salinity on leaf number was not significantly different from the second to the fourth week. However, by the fifth week, very high salinity (25 g/L NaCl) significantly reduced leaf number compared with non saline and moderate salinity treatments. Cakir et al. (2004) and Truşcă et al. (2023) found that salinity disrupts plants in many ways. High salinity holds back cell growth, makes it harder for seeds to sprout, cuts down on the water plants can use, bumps up cellulose in the leaves, and basically stunts leaf growth. When Na^+ levels go up to stomatal and chloroplast tissues, membranes get leakier, water potential drops, and stomata close up. Moreover, the respiration part, such as the electron transport chain bother and decreases photosynthesis rate (Assaha et al., 2017; Lin et al., 2025). An increasing salinity level causes a lower leaf area, stunted leaf growth, less light absorption, and a hindered photosynthesis rate (Manchanda & Garg, 2008; Majidian & Ghorbani, 2023). Principally, a saline condition causes osmotic pressure, which makes soybean water and nutrient disruption. Additionally, salinity triggers reactive oxygen species production in the cell. That leads to cell toxicity, damages membranes, and can end in cell death (Mo et al., 2020; Abulfaraj & Jalal, 2021).

Table 3 shows that there are no significant differences in combination of salinities and varieties in chlorophyll a content. Although not statistically significant, an upward trend in chlorophyll a was observed in Dering 1 as salinity concentrations increased. Chlorophyll a is the primary pigment involved in the photosynthesis process, and it is usually kept in higher amounts. According to Juwarno et al. (2018), salinity affected the content of chlorophyll a (might also be on chlorophyll b), the higher the salinity, the more chlorophyll a (and b) were present. Lenis et al. (2011), as cited in Newsome (2016), reported an increase in chlorophyll content under salt stress in some salt-tolerant Glycine accessions.

On the other hand, Table 4 shows that the combination of (5 g/L) (Moderate Saline) and Dering 3 shows a significant difference compared to the other chlorophyll b and total chlorophyll content, except for (15 g/L) (Very Saline) and Dering 1. Chlorophyll b is part of the light-harvesting complex and helps the plant adapt better to stressful conditions.

Chlorophyll b content increases at moderate salinity, then tends to decrease at very high salinity, except for Dering 1. At lower salt levels, soybeans can boost their chlorophyll levels, which helps improve their ability to perform photosynthesis. Chloroplast stability is only possible under conditions of moderate salinity. Adaptation mechanism of salinity stress Dering 3 and Wilis only up to (5 g/L) (Moderate Saline), and Dering 1 and Anjasmoro up to 15 g/L (Very Saline). Based on the reduction in chlorophyll levels, Anjasmoro and Dering 3 show the highest stability, Wilis demonstrates moderate stability, and Dering 1 shows the greatest sensitivity.

Salt stress greatly reduces the ability of plants to perform photosynthesis. This happens because salt affects the electron transport chain and causes oxidative damage, which harms the cell structure and the chlorophyll molecules. As a result, the metabolic processes within the plant are disrupted (Dai et al., 2024; Batool et al., 2026). The Na+ and Cl ions that accumulate in the chloroplast will inhibit photosynthesis because chlorophyll is the major photosynthesis pigment directly associated with plant growth and development (Saleem et al., 2020; Barus et al., 2023). The salinity-induced reductions in chlorophyll may be attributed to oxidative damage-induced membrane breakdown and chlorophyllase, which negatively impacts pigment synthesis (Aljuaid et al., 2022; Nasrallah et al., 2022; Qian et al., 2024).

Chlorophyll biosynthesis can be inhibited through a series of step-by-step reactions, resulting in a reduced chlorophyll level (Tanaka et al., 2006; Qian et al., 2024). Also, photosynthesis becomes disturbed by abiotic stress because damage to the enzyme connected with chlorophyll synthesis (Rajput et al., 2021; Alzahrani, 2024).

Several varieties exhibit stability in the configuration of total chlorophyll even in high saline conditions. The other varieties exhibit a significant decrease. This study finds that the interaction between salinity condition and variety as geneticly influence. Genetic character more affects the response of the plant to salt stress. There is a decline in total chlorophyll content caused by the salinity condition indicate negative impact on photosynthetic ability rather than each pigment composition. The inter-varietal variability in maintaining total chlorophyll indicates different levels of salinity tolerance, which can be the basis for selecting varieties tolerant to salt stress.

Salinity stress impacts obstacle on gas exchange, stomatal conductance capability, and electron transport optimization, thereby limiting the biosynthesis of chlorophyll. Chlorophyll degradation may be caused by ROS and essential nutrient transport configuration, as well as Na^{+} and Cl^{-} (Amjad et al., 2020; Alizadeh et al., 2024).

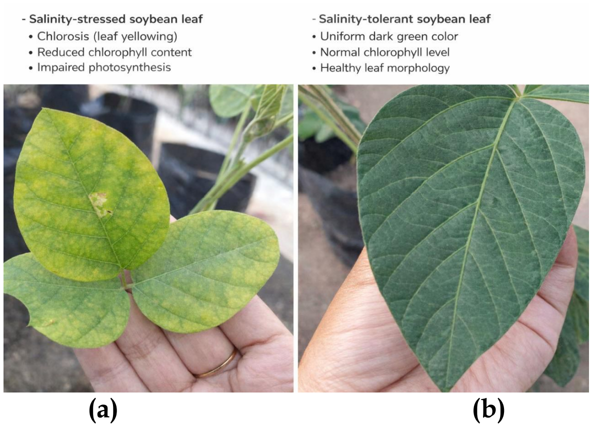


Figure 4. The chlorophyll expression as greenness of soybean leaves: a) Dering 1 (susceptible), b) Anjasmoro (resistant)

In the salinity condition, soybeans tend to be threatened, and this is reflected in leaf chlorosis, reduced growth, and lower biomass (Tomaškinová et al., 2025). Dering 1 exhibits obvious chlorosis symptoms, leaves characther able to changing color from pale green to yellowish. Chlorophyll content status indicates the pigment damage, especially chlorophyll a and total chlorophyll, because of biosynthesis disruption or degradation of chlorophyll under salinity conditions. Chlorosis as physiology reflection of a decrease in photosynthetic capability and pigment light-capturing efficiency, related to ionic and osmotic stress caused by the accumulation of Na^{+} and Cl^{-} ions in leaf tissues.

On the other hand, Anjasmoro leaves exhibit a dark green color with normal leaf shape morphology. This study exhibits relative stability of chlorophyll production, indicate photosynthethic capability undisturbed in saline conditions. These visual observations are consistent with the data for chlorophyll b and total chlorophyll, where Anjasmoro is able to

ISSN: 1412-4718, eISSN: 2581-138x

maintain higher chlorophyll levels rather than Dering 1 on various salinity treatments. Although it is technically at severity Dering 3 based on the data, in terms of phenotypic expression, Anjasmoro appears the greenest. This is suspected to be due to its leaf thickness and the structure of the leaf mesophyll tissue.

Overall, Dering 1 was more sensitive to salinity, indicated by an intense decrease in total chlorophyll and the appearance of chlorosis, while Anjasmoro exhibited salinity tolerance extensively, evident by its ability to maintain chlorophyll b and total chlorophyll content. Thus, the observed differences in chlorophyll expression in further research support the quantitative analysis by indicating that the photosynthetic response of soybeans to salinity is more influenced by the genetic factors of the variety.

Salt stress on plants tend indicated by chlorosis due to increased salt accumulation (Linh & Thang, 2021). The influence on the height of enzyme chlorophyllase activity under salinity stress, which leads to degraded chlorophyll accumulation, eventually causing leaf senescence and chlorosis (Rahman et al., 2016; Otie et al., 2021). Stress in the salinity condition depresses nitrogen nutrients in plants, this contribute in chlorophyll reduction (Khademian et al., 2019; Yaghoubian et al., 2021).

Table 5 shows that there is no interaction between salinities and varieties in yield parameters. Soybeans may grow in saline conditions. Growth is regarded as successful when it reaches the highest stage of its life cycle, which is the generative phase. Soybeans use a variety of strategies to deal with stress. Flowering time generally did not exhibit any delay among the tested varieties. All varieties, except Dering 1, showed no significant differences in mean flowering time. The pod formation stage, Dering 1 and Dering 3 did not differ significantly from each other; however, both varieties differed significantly from the remaining varieties. Regarding the number of pods per plant, Dering 3 produced the highest number of pods. This value was not significantly different from those of Anjasmoro and Wilis, but it was significantly higher than that of Dering 1. In terms of seed number, Anjasmoro produced the highest number of seeds and did not differ significantly from Wilis and Dering 3, whereas it differed significantly from Dering 1. The weight of 100 seeds is generally low across all varieties. Anjasmoro is not significantly different from Wilis and Dering 3, but shows a significant difference compared to Dering 1. Harvest age average has no significant difference among varieties. Anjasmoro, Wilis, Dering 1, and Dering 3 reach harvest time between 87 and 90 days after planting (DAP), these results are still close to the respective variety descriptions under normal conditions. Salinity stress basically affected the yield components more than the harvest time. During the generative phase, Anjasmoro and Wilis mostly exhibited greater stability compared with Dering 1 and Dering 3. This condition indicates adaptation of the generative stage following NaCl treatment at 30 and 50 DAP.

Table 5. Flowering time, pod forming time, number of pods, number of seeds, weight of 100 seeds, and harvest time of superior soybean (*Glycine max* L.) varieties from East Java on several levels of salinity

	Flowering Time	Pod Forming Time	Number of Pods	Number of Seeds	Weight of 100 Seeds	Harvest Time
	DAP	DAP	pod	seed	g	DAP
Varieties						
V1 (Anjasmoro)	43.17a	54.25a	44.83a	82.83a	4.80a	88.1ab
V2 (Wilis)	43.75a	54.83a	27.25b	56.50ab	4.07a	87.0ab
V3 (Dering 1)	50.75b	64.17b	7.17c	5.08b	0.07b	90.0b
V4 (Dering 3)	44.33a	61.25b	37.50ab	80.92a	1.75b	90.0b
Salinities (NaCl)						
S0 (0 g/l) (Non Saline)	47.83	58.92	45.67a	106.17a	3.22	88.8
S1 (5 g/l) (Moderate Saline)	43.75	57.75	33.67ab	60,42ab	3.39	88.6
S2 (15 g/l) (Very Saline)	44.10	57.56	21.33bc	34.50b	2.72	89.1
S3 (25 g/l) (Very High Saline)	44.10	56.78	16.08c	24.25b	1.36	88.9

Note: Means followed by the same letter in the table are not significantly different at 5% DMRT.
DAP: Days After Planting

Salinity as a single factor did not significantly affect flowering time, pod emergence time, 100-seed weight, or harvest time. However, the number of pods and seeds per plant is significantly different between non-saline and high-salinity conditions.

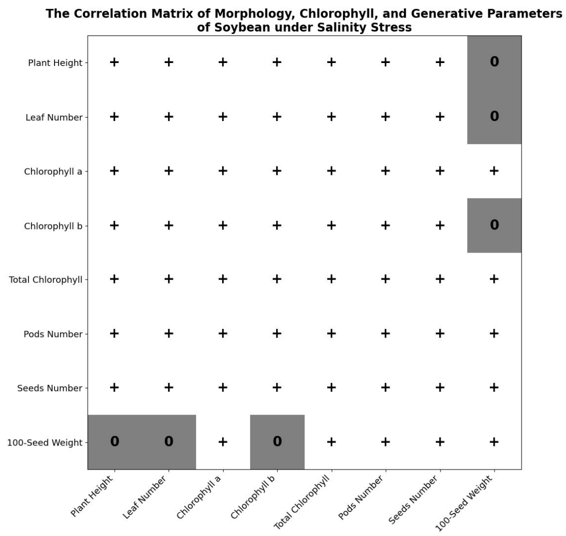
Salinity stress reduces soybean growth parameters, pod number per plant, and yield by disrupting nutrient balance and plant water relations (Essa, 2002; Tareq et al., 2022). In this study, a reduction in the number of pods at 26.3-64.8% and the number of seeds at 43.1-77.2%. The weight of 100 seeds was reduced by 15.5-57.8% in very and very high saline conditions. Seed number is the most sensitive parameter to salinity stress.

Salt stress also affects essential physiological and metabolic systems in plants, such as protein synthesis, nutrient and water absorption, assimilate translocation, and cytosolic and mitochondrial responses. These abnormalities result in less biomass accumulation and reduced reproductive development (Alharby et al., 2021; Toshtemirovna & Raufovna, 2025).

The differences in the varieties are the factor was linked to genetically influence that are genetically influenced and affect plant adaptation, nutrient uptake, and how well it can handle stress from the environment. The impact of the salinity factor tends in higher salinity levels head to slow down plant growth in higher salinity levels. Since they cause ionic imbalance, lower osmotic tension, and disruption of photosynthesis. Observations indicate a single factor is able to control plant response, even without significant interaction between the combination factors. Therefore, every single factor is considered an important parameter in the evaluation of plant growth and stress tolerance.

Salinity is one of the abiotic stressors that is able to limit crop productivity by affecting plant growth until generative. Plants modify many resistance mechanisms in response to salt stress periods (Asif et al, 2014; Kang et al., 2023). Sensitivity to salinity in soybean peaks during flowering and the early stages of grain development (Kondetti et al., 2012; Pavli et al., 2021). Salinity stress adversely affects soybean growth, seed quality and quantity, and overall yield (Khan et al., 2019; Khan et al., 2021).

The most vegetative and physiological parameters exhibit positive relationships with generative traits. Total chlorophyll shows the strongest and most closely positive association with yield components, though the relationship between vegetative growth traits and 100 seed weight appears weak. The relationship between the weight of 100 seeds and some parameters cannot be established. Chlorophyll b barely changed across all salinity treatments. Reduction in chlorophyll b was not significantly affected even under high saline conditions. The weight of 100 seeds really depends on photosynthesis yield availability during the seed-filling stage. Therefore, it shows a stronger association with chlorophyll a than with chlorophyll b.



+: Positive correlation o: No correlation
(Pearson's correlation coefficient (Pearson, 1896))

Figure 5. The Correlation matrix of chlorophyll, morphology, and generative parameters of soybean under salinity stress

Saline condition desructive on gas exchange activities, photosynthetic component, and nutrient transportation, eventually decreases yield component. It also degrades chlorophyll pigments, both chlorophyll a and chlorophyll b (Pan et al., 2021; Shahriar-Tareq et al., 2021).

In general, every single factor indicates different genes among soybean varieties, as revealed in saline level consistently. Salinity plays a role as a main effect in a single significant effect on generative character, tend increasing salt levels uniformly reduce plant performance regardless of variety.

Anjasmoro was identified as a superior soybean variety from East Java that was evaluated in this study and found to be the most

ISSN: 1412-4718, eISSN: 2581-138x

salt-tolerant. This variety constantly indicates more stable generative and physiological parameters under salinity stress. Resistance trait is evident in tolerance mechanism till very high salinity conditions. However, further observation on genetic analysis is needed to explain the main mechanisms of this salt tolerance.

Ion homeostasis is a key mechanism underlying salt tolerance in soybeans, and major genes such as GmCHX1 in the soybean genome have been validated. As a result, targeting ion transport processes is one of the most effective strategies for developing or selecting salt-tolerant cultivars (Leung et al., 2023). At the side genetic factor, abiotic management improvement through technology inovation as important factor. Plants resilient under stress conditions use management strategies of exogenous agronomic treatment.

Conclusion

Based on chlorophyll content, morphological parameters, and yield, Anjasmoro was identified as the most salt-tolerant soybean variety. Tolerance rate indicates high to very high salinity levels (15–25 g L^{-1} NaCl) by the stability of the number of pods, seed number, 100 seeds weight, chlorophyll a, and total chlorophyll. The salinity resistance order is: Anjasmoro, Dering 3, Wilis, and Dering 1.

Acknowledgments

The author sincerely thanks to LPPM UPN "Veteran" Jawa Timur for supporting this research through the 2023 Flagship Research funding scheme.

References

Açıkbaş S, Özyazıcı M.A., Bıçakçı E, Özyazıcı G. 2023. Germination and seedling development performances of some soybean (*Glycine max* (L.) Merrill) cultivars under salinity stress. Turkish Journal of Range and Forage Science, 4: 108-118.

Aboh AA, Eyong, OI. 2023. Effects of Salinity Stress on Growth Parameters of Soybeans (*Glycine max*) Cultivated in Obubra Local Government Area, of Cross River State Nigeria. Asian Journal of Research in Crop Science, 8: 465-470.

Abulfaraj AA, Jalal RS. 2021. Use of Plant Growth-Promoting Bacteria to Enhance Salinity Stress in Soybean (*Glycine max* L.) plants. Saudi journal of biological sciences, 28: 3823-3834.

Alam P, Arshad M, Al-Kheraif AA, Azzam MA, Al Balawi T. 2022. Silicon nanoparticle-induced regulation of carbohydrate metabolism, photosynthesis, and ROS homeostasis in Solanum lycopersicum subjected to salinity stress. ACS omega, 7: 31834−31844.

Alizadeh MM, Gerami M, Majidian P, Ghorbani HR. 2024. The Potential Application of Biochar and Salicylic Acid to Alleviate Salt Stress in Soybean (*Glycine max* L.). Heliyon, 10: 1-16.

Alzahrani Y. 2024. Evaluation of drought and salinity tolerance potentials of different soybean genotypes based upon physiological, biochemical, and genetic indicators. Frontiers in Plant Science, 15: 1-13.

Batool M, El-Badri AM, Zheng L, Wang C, Wang Z, Ikram M, Ullah M, Ikram M, Waqas M, Kuai J, Zhang C. 2026. Comprehensive Morpho-Physiological Responses Underlying Salt Tolerance at Different Time Points in Brassica napus Seedlings. Plants, 15: 1-21.

Barus WA, Sulistiani R, Utami S, Tarigan DM, Lestami A. 2023. Black Soybean Response to Antioxidant Application for Growth and Yield under Salinity Stress Conditions. SABRAO Journal of Breeding and Genetics, 55: 417-426

Bougrine H, Hadjout S, Zouidi M, Belkendil A, Zeghmar A, Boulekdam C, Ouaret W, Soufan W, Belhouadjeb FA, Mebarkia A. 2025. Assessment of Salinity Tolerance and Ecotypic Variability in *Vicia narbonensis* L.: Morphological, Physiological, and Biochemical Responses. Phyton, 94: 251-267.

Colin L, Ruhnow F, Zhu JK, Zhao C, Zhao Y, Persson S. 2023. The cell biology of primary cell walls during salt stress. Plant Cell, 35: 201-217.

Dong T, Yan L, Wang J, Niu Y, Wang L. 2025. Salt Tolerance in Soybean (*Glycine max* L.): A Comprehensive Review of Molecular Mechanisms, Key Regulators, and Future

Perspectives for Saline Soil Utilization. Plants, 14: 1-21.

Han L, Ge L, Fei L, Huang C, Li Y, Fan W, Zhu D, Zhao L. 2025. A Comprehensive Evaluation of Soybean Germplasm Resources for Salt Tolerance During Germination. Plants, 14: 1-16.

Hasanuzzaman M, Parvin K, Anee TI, Masud AAC, Nowroz F. 2022. Salt Stress Responses and Tolerance in Soybean. In Plant stress physiology-perspectives in agriculture. IntechOpen. London.

Jha S, Singh J, Chouhan C, Singh O, Srivastava RK. 2022. Evaluation of multiple salinity tolerance indices for screening and comparative biochemical and molecular analysis of pearl millet [*Pennisetum glaucum* (L.) R. Br.] genotypes. Journal of Plant Growth Regulation, 41: 1820-1834.

Juwarno J, Suparjana TB, Abbas M. 2018. Mahameru soybean (*Glycine max*) cultivar, high salinity tolerant. Biosaintifika: Journal of Biology & Biology Education, 10: 23-31.

Kang SM, Hoque MIU, Woo JI, Lee IJ. 2023. Mitigation of Salinity Stress on Soybean Seedlings Using Indole Acetic Acid-Producing Acinetobacter Pittii YNA40. Agriculture, 13: 1-15.

Khan MA, Sahile AA, Jan R, Asaf S, Hamayun M, Imran M, Adhikari A, Kang SM, Kim KM, Lee IJ. 2021. Halotolerant Bacteria Mitigate The Effects of Salinity Stress on Soybean Growth by Regulating Secondary Metabolites and Molecular Responses. BMC Plant Biology, 21: 1-15.

Kokebie D, Enyew A, Masresha G, Fentie T, Mulat E. 2024. Morphological, physiological, and biochemical responses of three different soybean (*Glycine max* L.) varieties under salinity stress conditions. Frontiers in Plant Science, 15: 1-14.

Leung HS, Chan LY, Law CH, Li MW, Lam HM. 2023. Twenty years of mining salt tolerance genes in soybean. Molecular Breeding, 43: 1-17.

Lin T, Haider FU, Liu T, Li S, Zhang P, Zhao C, Li X. 2025. Salt Tolerance Induced by Plant Growth-Promoting Rhizobacteria Is Associated with Modulations of the Photosynthetic Characteristics, Antioxidant System, and Rhizosphere Microbial Diversity in Soybean (*Glycine max* (L.) *Merr*.). Agronomy, 15: 1-18.

Linh NT, Thang VN. 2021. Effects of salinity stress on the growth, physiology, and yield of soybean (*Glycine max* (L.) Merrill). Vietnam Journal of Agricultural Sciences, 4: 1043-1055.

Mahajan M, Poor P, Kaur H, Aher RR, Palakolanu SR, Khan MIR. 2025. Salt stress tolerance and abscisic acid in plants: associating role of plant growth regulators and transcription factors. Plant Physiology and Biochemistry, 228: 1-13.

Majidian P, Ghorbani H. 2024. Salinity stress in plants: challenges in view of physiological aspects. In Abiotic stress in crop plants-ecophysiological responses and molecular approaches. IntechOpen. London.

Mousavi SS, Karami A, Maggi F. 2022. Photosynthesis and chlorophyll fluorescence of Iranian licorice (*Glycyrrhiza glabra* L.) accessions under salinity stress. Frontiers in Plant Science, 13: 1-19.

Narayana RC, Anwar S, Karno K. 2024. Respons Morfologi dan Produksi Tanaman Kedelai (*Glycine max* L.) terhadap Penggunaan Berbagai Dosis Asam Salisilat dalam Cekaman Salinitas. Buana Sains, *23*: 107-116.

Nasution SN, Ghulamahdi M, Melati M. 2024. Impact of salinity stress on soybean growth and yield under saturated soil culture in tidal lands: A comparative study of tolerant varieties. Journal of Tropical Crop Science, 11: 287-298.

Newsome JA. 2016. Dissecting Salt Tolerance in Soybean by Profiling Differential Physiological Responses under Salt Stress. University of Arkansas. US.

Otie V, Udo I, Shao Y, Itam MO, Okamoto H, An P, Eneji EA. 2021. Salinity Effects on Morpho-Physiological and Yield Traits of Soybean (*Glycine max* L.) as Mediated by Foliar Spray With Brassinolide. Plants, 10: 1-22.

Pavli OI, Foti C, Skoufogianni G, Karastergiou G, Panagou A, Khah, E.M. 2021. Effect of Salinity on Seed Germination and Seedling Development of Soybean Genotypes. Int J Environ Sci Nat Res., 27: 1-9.

Pearson K. 1896. Mathematical contributions to the theory of evolution. III. Regression, heredity, and panmixia. Philos Trans R Soc Lond A, 187: 253-318.

Peng Y, Zhu H, Wang Y, Kang J, Hu L, Li L, Zhu K, Yan J, Bu X, Wang X, Zhang Y. 2025. Revisiting the role of light signaling in plant responses to salt stress. Horticulture Research, 12: 1-11.

ISSN: 1412-4718, eISSN: 2581-138x

Pujiwati H, Suharjo UK, Prameswari W, Husna M, Gonggo B, Ginting S, Susilo E. 2021. Morphological and physiological performances of 18 soybean varieties exposed to salinity stress. J. Agron. Indonesia, 49: 251-258.

Qian Z, Lu L, Zihan W, Qianyue B, Chungang Z, Shuheng Z, Jiali P, Jiaxin Y, Shuang Z, Jian, W. 2024. Gamma-aminobutyric acid (GABA) improves salinity stress tolerance in soybean seedlings by modulating their mineral nutrition, osmolyte contents, and ascorbate-glutathione cycle. BMC Plant Biology, 24: 1-18.

Sackey OK, Feng N, Mohammed YZ, Dzou CF, Zheng D, Zhao L, Shen X. 2025. A comprehensive review on rice responses and tolerance to salt stress. Frontiers in Plant Science, 16: 1-26.

Sathiyamurthi S, Saravanan S, Karuppannan S, Balakumbahan R, Sivasakthi M, Praveen Kumar S, Ramya M, Hussain S, Tariq A. 2024. Agricultural land suitability of Manimutha Nadhi watershed using AHP and GIS techniques. Discover Sustainability, 16: 1-20.a

Shahriar-Tareq M, Mannan MA, Al Mamun MA, Hossain MS, Higuchi H, Karim MA. 2021. Salinity induced changes in growth and physiology of field crops. Bangladesh Journal of Ecology, 3: 1-9.

Sultan MT, Mahmud U, Khan MZ. 2023. Addressing soil salinity for sustainable agriculture and food security: Innovations and challenges in coastal regions of Bangladesh. Future Foods, 15: 1-16.

Tanveer M, Mahmood A, Alawadi HFN, Adiba A, Javaid MM, Khan BA, Wahid A, Abdullah F, Seleiman MF. 2025. Impact of boron on Glycine max L. to mitigate salt stress by modulating the morpho-physiological and biochemical responses. BMC Plant Biology, 25: 1-21.

Tareq MS, Mannan MA, Rahman MM, Mamun MAA, Karim MA. 2022. Salinity-induced changes in growth, physiology and yield of soybean genotypes. Annals of Bangladesh Agriculture, 26: 29-48.

Tomaškinová J, Tomaškin J, Drimal M, Bellido J. 2025. The Impact of Abiotic Environmental Stressors on Fluorescence and Chlorophyll Content in *Glycine max* (L.) Merrill. Agronomy, 15: 1-18.

Toshtemirovna NU, Raufovna KG. 2025. The Effect Of Salinity On The Growth and Development of Soybean Varieties. Modern American Journal of Biological and Environmental Sciences, 1: 78-87.

Truşcă M, Gâdea Ş, Vidican R, Stoian V, Vâtcă A, Balint C, Stoian VA, Horvat M, Vâtcă S. 2023. Exploring the Research Challenges and Perspectives in Ecophysiology of Plants Affected by Salinity Stress. Agriculture, 13: 1-19.

Van Zelm E, Zhang Y, Testerink C. 2020. Salt tolerance mechanisms of plants. Annual review of plant biology, 71: 403-33.

Vogel JT, Liu W, Olhoft P, Crafts-Brandner SJ, Pennycooke JC, Christiansen N. 2021. Soybean yield formation physiology–a foundation for precision breeding based improvement. Frontiers in plant science, 12: 1-15.

Yaghoubian I, Ghassemi S, Nazari M, Raei Y, Smith DL. 2021. Response of Physiological Traits, Antioxidant Enzymes and Nutrient Uptake of Soybean to Azotobacter Chroococcum and zinc sulfate under salinity. South African Journal of Botany, 143: 42-50.

ISSN: 1412-4718, eISSN: 2581-138x

Putri SD · Balapradhana AK · Ariyanti M · Anjarsari IRD · Maxiselly Y

The effect of liquid foliar treatments and pruning combinations on the growth of productive phase arabica coffee plants (*Coffea arabica* L.)

Abstract. In recent years, productivity decline in arabica coffee has become a real issue in Indonesia. This decay has been brought about by various external and internal factors that are beyond human control. Various factors, including suboptimal cultivation practices, take part in this process. The objective of the present study was to evaluate the effect of pruning combined with foliar fertilizer application (coconut water and sugarcane molasses) and 6-benzylaminopurine (BAP) on the vegetative growth of arabica coffee plants during the production phase. The research was conducted at Ciparanje Experimental Field, Universitas Padjadjaran, using a randomized complete block design with seven treatments including a control (without pruning and foliar application), application of coconut water (75%, 750 mL L^{-1}), sugarcane molasses (2 mL L^{-1}), and BAP at 60 ppm, each applied with and without pruning, and four replications. In this research, the main parameters observed included the number and length of the lateral branches, leaf chlorophyll index, leaf area, canopy width, number of fruit clusters per branch and per tree, and coffee cherry fruit weight. It emerged that the combination of pruning and the use of sugarcane molasses at 2 mL L^{-1} significantly increased lateral branch formation, leaf area, and the number of fruit clusters per tree. The integration of pruning with foliar treatments effectively supported optimal growth of productive-phase arabica coffee plants.

Keywords: 6-benzylaminopurine · Coconut water · Coffee cherry · Lateral branches · Sugarcane molasses

Submitted: 13 February 2026, Accepted: 31 March 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69851

Putri SD[1] · Balapradhana AK[1] · Ariyanti M[2] · Anjarsari IRD[2] · Maxiselly Y[2]*
[1]Agrotechnology Program, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia
[2]Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21 Jatinangor, Sumedang 45363, Indonesia
*Correspondence: yudithia.maxiselly@unpad.ac.id

Putri SD, Balapradhana AK, Ariyanti M, Anjarsari IRD, Maxiselly Y. 2026 The effect of liquid foliar treatments and pruning combinations on the growth of productive phase arabica coffee plants (*Coffea arabica* L.). Jurnal Kultivasi, 25 (1): 87-99.

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Arabica coffee is one of the most significant Indonesian coffee commodities. This implies that the product makes a substantial contribution to agricultural income for the country. According to the United States Department of Agriculture (2024), Indonesia placed 16th globally regarding the production of arabica coffee. Thus, this shows the contribution of the Indonesian coffee Industry to the global economy. However, this is also paired with the significant potential for growth, i.e., increased demand for the product globally. Looking at the national levels of the industry, it has been observed that there has been a decline in the production of the product over the recent past. Data from the United States Department of Agriculture (2024) indicate a reduction from 78,000 tons of arabica coffee produced in 2020/2021 to 72,000 tons of the product from 2022/2023. This shows that there are still various issues to be solved related to the management of the plants.

The reduction of productivity for arabica coffee is not solely explained by land changes but is equally shaped by daily field-level farming activities. Fertilization and pruning activities for coffee farms, which contain arabica coffee, occur using traditional methods and not technology in most areas of Indonesia (Rico et al., 2021). This implies that fertilization and pruning take place in a poor manner relative to what is required for the development of coffee plants. Fertilizers play a vital role in ensuring the provision of substances required for the formation of coffee leaves or fruits (Ma et al., 2022). Pruning of coffee plants is effective for maintaining illumination within the plant and influencing the direction of plant development (Mohammed & Worku, 2025; Sharma et al., 2025).

Additionally, and most importantly, if fertilization and pruning are carried out properly, the actual effect of these two activities on the further improvement of the coffee plants becomes unmistakable. Previous studies revealed that the integration of pruning and fertilizer has positive effects on the cultivation management of arabica coffee plants. Integration of alternative pruning methods and fertilizer, either organic or inorganic, promotes the stem diameter, shoots, chlorophyll content, and soil qualities (Rohani et al., 2024). Pruning has been found to have an effect on the microclimate and the yield of the plants, and fertilization on the anatomy and physiological properties of the leaves (Muliasari et al., 2021; Soares et al., 2022).

Most of the coffee farmers in Indonesia still apply inputs directly to the soil, due to the fact that most of the necessary ones are NPK and urea, which are easy to obtain and easy to use. However, under field conditions, a large amount of these nutrients is usually lost through processes such as leaching, fixation by the soil, or competition with weeds, thus lowering nutrient availability for the plants (Asare et al., 2023; Barłóg et al., 2022; Little et al., 2021; Mao et al., 2024). In such a case, it is increasingly important to adopt more efficient fertilizer methods while ensuring sustainability.

One alternative fertilization that would help solve the problems stated above is foliar fertilization. The fertilizers would be used to provide nutrients to the leaves, which can be easily absorbed by the plant (Arsic et al., 2022; Niu et al., 2020). The materials that can be used as foliar fertilizers are sugarcane molasses and coconut water, and 6-benzylaminopurine (BAP) as a plant growth regulator. BAP makes use of cytokinin, which stimulates cell division and shoot development (Ali et al, 2021; Zarea & Karimi, 2023). On the other hand, molasses and coconut make use of nutrients as well as growth regulators, which improve plant metabolism and development (Salman, 2022; Stephen et al, 2024). The materials applied in this study are generally obtainable, although their availability and cost-effectiveness can differ across locations. Coconut water and molasses may represent relatively inexpensive inputs in some areas, while the inclusion of BAP, a synthetic plant growth regulator, may require additional investment depending on usage and sourcing. Consequently, the economic implications of these treatments depend on the location and were not the primary focus of this study.

Apart from fertilization, pruning is another significant factor to be followed during the cultivation of Coffee. Thus, pruning is a procedure to control the height of the plant, allow penetration of sufficient light, avoid pests, and prevent plant infection (Gokavi et al., 2021; Rahman et al., 2024). The application of pruning during the fruiting stage improves the number of fruitful branches and the photosynthetic surface area (Yang et al., 2023; Zhang et al., 2022). In relation to this, the explanation from the physiological perspective of pruning is essential for leaf and fruit development, which determines the productivity of the crop (Dong et al., 2019; Colodetti et al., 2020).

The combination of pruning and the application of liquid fertilizers has emerged as a potential approach for enhancing the productivity of arabica coffee varieties. It is observed that the sensitivity response takes place during pruning, which starts the healing process for the affected part of the plant, hence the need for increased nutrition. At this point, foliar fertilizers become significant in the recovery process, as they can provide the necessary nutrients for the development of new shoots (Xie et al., 2023). Aside from plant physiology, the combined effects of pruning and fertilization also have significant effects on soil. Rowe et al. (2022) found that these practices not only affect coffee yield but also impact environmental services like soil carbon and nitrogen content. Furthermore, Kurniawan et al. (2024) reported that pruning also increased soil total nitrogen content by 10-56% compared with unpruned trees. At the production level, Siahaan et al. (2020) found that the combined effects of pruning and fertilization significantly increased the number of productive branches, canopy diameter, fruit clusters, fruits per cluster, and seed weight. However, despite these positive results, studies that specifically examine the combined effects of these two practices are still relatively limited, highlighting the need for further research.

Based on these conditions, further research was needed on the integration of foliar treatments and pruning in Arabica coffee cultivation. Therefore, this study aimed to evaluate the effects of liquid foliar application and pruning on the growth and productivity of Arabica coffee plants during the production phase and to determine the most effective treatment combination for increasing yield. It was expected that the appropriate combination of foliar treatments and pruning, particularly through the use of molasses-based formulations and suitable pruning techniques, could improve plant growth and yield performance.

Materials and Methods

The experiment was conducted at the Ciparanje Experimental Field, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, from December 2024 to April 2025, at an altitude of approximately 730 m above sea level. The plant material consisted of three-year-old arabica coffee plants of the Lini S 795 variety in the productive phase, grown at the experimental field. Materials used in this study included coconut water, sugarcane molasses, and liquid Benzyl Amino Purine (BAP). Other tools used in this experiment include hoes, pruning shears, watering cans, machetes, measuring tapes, vernier callipers, buckets, plant labels, stationery, a hand sprayer, and documentation tools. This study was arranged in a randomized complete block design (RCBD) with seven treatments and four replications. Each experimental unit contained two plants, making a total of 56 plants for treatment. The treatments applied were: (A) control without pruning and foliar application; (B) 75% of coconut water (750 mL L^{-1} water) with pruning; (C) 2 mL L^{-1} water sugarcane molasses with pruning; (D) 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; (E) 75% of coconut water without pruning; (F) 2 mL L^{-1} water sugarcane molasses without pruning; and (G) 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

SPSS software was used to analyse the data obtained from the field experiments, considering 5 percent significance level for analysis of variance. The F-test at a probability level of 5 percent was used to test the significance of treatment effects. Then, when significant differences between treatment means occurred, DMRT was used at the 5 percent level of significance for further mean comparisons.

The research started by uprooting weeds and other remnants of old plants from around the plant units. The young coconut water used in this research was extracted from selected coconuts sourced from local markets, approximately seven to eight months after flowering, selected due to the smoothness of their green outer shells. This was collected in clean containers and filtered through a fine sieve to eliminate debris and any other impurities, and immediately applied to minimize nutrient degradation. In addition, the sugarcane molasses used in this experiment was a liquid fertilizer based on molasses (liquid waste sugarcane). Moreover, the 6-benzylaminopurine (BAP) used in this study was a synthetic cytokinin commercially available under the trade name 6-BAP.

Pruning was carried out at the beginning of the experiment (0 weeks) to maintain an average plant height of 153 cm above ground level (Rodrigues et al., 2017). The Arabica coffee plants used in this research were approximately three years old and were uniformly pruned within each experimental unit. The first foliar application was conducted immediately after pruning (0 weeks), followed by subsequent applications at 2, 6, 8, and 10 weeks after pruning. Foliar treatments involve using a hand sprayer, covering all parts of the plants, including the top and bottom parts of leaves. Spraying the leaves was conducted until they were evenly covered to ensure an even spread of the substance and its even absorption. Observed variables included the increased number of lateral branches, increased elongation of lateral branches, chlorophyll index, leaf area, width of the canopy, number of fruit clusters on the branch, number of fruit clusters on the plants, and the weight of the fruit.

Results and Discussion

Number of Lateral Branches. BAP treatment at 60 ppm, coupled with pruning, showed substantial improvement in lateral branches (Table 1). Based on Table 1, the application of liquid foliar treatments under different treatments did not significantly affect the development of lateral branches of arabica coffee plants at 2 to 6 weeks after treatments (WAT). However, a significant response began to appear at 8-12 WAT. At this point, the combination of BAP at 60 ppm and pruning gave a higher increase in the number of lateral branches than the other treatments. Although this increase was not significantly different from the 75% coconut water with pruning treatment and the 2 ml L^{-1} sugarcane molasses with pruning treatment, the combination of BAP at 60 ppm and pruning had the highest mean value.

This response is presumed to be related to the effect of pruning, which removes the apical growing point and alters the distribution of photosynthates within the plant. As a result, assimilates are redirected to other organs, particularly lateral branches (Muliasari et al., 2021). Physiologically, pruning stimulates the activation and extension of lateral branch growth. The change in the distribution of photosynthate allocation due to pruning means that there is more scope for the further development of lateral branches optimally. Damayanti et al. (2022) and Postma et al. (2020) found that reduction of vegetative parts could reduce competition of inner parts to get enough nutrients, enabling each branch to get enough nutrients. Besides, breaking of apical dominance is strongly related to a favourable condition for the induction of a lateral meristematic growth, which can also be triggered by chemical substances.

The impact of pruning with foliar treatments spraying has shown its efficiency to boost activity for the lateral meristem. This further activates cytokinin, a plant growth hormone present in BAP and coconut water, to effectively induce lateral branch development. The spraying of LPG, i.e., BAP, and coconut water, which is rich in cytokinin, is of significant importance in speeding up the division and differentiation of plant cells, which is a key factor towards photosynthesis, thus being essential to maintain healthy plants (Ikeda et al., 2021).

Table 1. Average increase in number of lateral branches of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in the Number of Lateral Branches					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	1.24	1.81	2.07	2.25 b	2.33 c	2.35 c
B	1.25	1.44	2.31	2.71 ab	3.05 ab	3.20 ab
C	1.11	1.53	2.52	2.80 ab	3.05 ab	3.17 ab
D	1.24	1.47	2.50	3.21 a	3.63 a	3.82 a
E	1.25	1.76	1.94	2.21 b	2.48 bc	2.55 bc
F	1.11	1.96	2.38	2.74 ab	2.97 abc	3.04 bc
G	1.38	1.52	2.13	2.43 b	2.67 bc	2.73 bc
CV	18.01%	18.05%	17.29%	15.83%	14.88%	14.72%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

The presence of sugarcane molasses acts as a source of phosphorus and nitrogen, which play a significant role in the improvement of plant metabolism. Higher chlorophyll content may improve photosynthetic efficiency, resulting in increased assimilate production, which is of critical importance for plant development, especially towards branch development (Busato et al., 2022).

Of all the treatments, 60 ppm BAP with pruning gave the best stimulation in terms of the elongation of the lateral branches and plant development. Such a result indicates that the suitable integration of pruning and cytokinin-based foliar treatments creates favourable physiological conditions for the development of lateral branches, thereby improving growth performance in arabica coffee plants.

Lateral Branch Length. The application of sugarcane molasses at a concentration of 2 ml L^{-1} showed a significant effect on the increase in lateral branch length of productive arabica coffee plants at 8 to 12 WAT (Table 2). Table 2 shows that the treatment of sugarcane molasses at 2 ml L^{-1} combined with pruning was significantly different from the treatments of 75% coconut water without pruning, BAP at 60 ppm without pruning, and the control. Meanwhile, the BAP treatment at 60 ppm without pruning did not differ significantly from the control during the observation period from 8 to 12 WAT. Among all treatments, sugarcane molasses at 2 mL L^{-1} combined with pruning resulted in the greatest increase in lateral branch length (Table 2).

The increase in branch length was strongly dependent on pruning treatment. Pruning influences the distribution of the pattern of photosynthates in the plant by diverting the focus of energy accumulation from the main shoot towards other organs, such as lateral branches, thereby giving these organs priority in growth. According to Muzaiyyanah et al. (2020), during the vegetative and generative growth phases, photosynthates are distributed and accumulated across various plant organs.

Photosynthates are essential for providing the energy required for the development of plant organs (Igamberdiev & Bykova, 2022). The allocation of photosynthetic products can be regulated by cutting the plant, enabling targeted allocation to organs known to be more actively developing, especially lateral branches (Yang et al., 2023; Zhong et al., 2024). Moreover, the sugarcane molasses used in this study contain macronutrients, including nitrogen (minimum 4%), phosphorus (minimum 10%), and potassium (minimum 8%), as well as micronutrients such as manganese (maximum 0.25%), zinc (maximum 0.25%), boron (maximum 0.125%), copper (maximum 0.25%), molybdenum (maximum 0.001%), and cobalt (maximum 0.0005%) which are important for plant growth (Stephen et al., 2024). Phosphorus and potassium in molasses fertilizer are significant in cell division and elongation in branch apical meristem tissues (Vinecky et al, 2017). These physiological processes are related to lateral branch development.

Table 2. Average increase in lateral branch length of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Lateral Branch Length (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	1.29	1.63	2.46	1.86 bc	2.14 cd	2.19 c
B	1.26	1.62	2.61	2.02 ab	2.52 ab	2.83 b
C	1.49	1.89	3.43	2.30 a	2.84 a	3.33 a
D	1.63	1.74	2.64	2.00 ab	2.43 bc	2.72 b
E	1.33	1.52	2.69	1.49 c	2.11 cd	2.83 b
F	1.42	1.78	2.96	2.15 ab	2.63 ab	2.73 b
G	1.40	1.57	2.44	1.79 bc	1.85 d	1.87 c
CV	14.41%	19.02%	16.77%	12.19%	10.15%	10.70%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

ISSN: 1412-4718, eISSN: 2581-138x

Of all the treatments, sugarcane molasses solution at a concentration of 2 ml L⁻¹, when used in combination with pruning, was found to be the most effective for promoting the increase in lateral branch length. This finding suggests that combining pruning with foliar fertilization with a sugarcane molasses solution promotes specific physiological conditions. Pruning may enhance assimilate redistribution toward lateral branches as dominant sinks, allowing more efficient utilization of nutrients absorbed through foliar application to support their growth (Jing et al., 2018).

Leaf Chlorophyll Index. The results showed that no significant differences in chlorophyll index were observed among treatments at all observation times (Table 3). This indicates that the application of foliar treatments and pruning did not significantly influence chlorophyll content under the conditions of this study.

However, slight variations observed at early stages may be associated with the rapid absorption of nutrients through the stomata and cuticle, which can temporarily enhance physiological activity following treatments such as pruning. Over time, plant responses to foliar application may become less pronounced as plants enter a more stable physiological phase, reducing variation among treatments (Henningsen et al., 2022). In addition, chlorophyll content is influenced by environmental and physiological factors, including light availability within the canopy and leaf age. Increased light exposure after pruning may support chlorophyll synthesis, while older leaves tend to have a higher chlorophyll index due to physiological maturity (He et al., 2019). Coconut water contains natural plant growth regulators such as cytokinins and auxins, which are reported to support chlorophyll synthesis and delay leaf senescence (Hönig et al., 2018; Shayantavi et al., 2025). However, in this study, their application did not result in a statistically significant effect, suggesting that environmental factors or plant physiological conditions may have played a more dominant role.

Table 3. Average increase in leaf chlorophyll index of productive arabica coffee at 4, 8, and 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Leaf Chlorophyll Index		
	4 WAT	8 WAT	12 WAT
A	55.2	58.9	64.2
B	53.6	60.1	70.9
C	55.3	60.0	71.1
D	57.0	64.5	70.5
E	52.2	58.1	65.5
F	52.8	56.6	60.2
G	54.5	59.9	66.8
CV	6.09%	5.96%	8.22%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L⁻¹ water) with pruning; C= 2 mL L⁻¹ water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L⁻¹ water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L⁻¹ water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L⁻¹ water) liquid BAP without pruning.

Table 4. Average increase in leaf area of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Leaf Area (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	2.46	2.75	3.06	3.17 d	3.95 e	3.95 d
B	2.37	2.78	3.23	6.65 a	8.01 a	8.07 a
C	2.28	2.45	3.00	3.24 d	5.99 bc	6.95 b
D	2.59	3.30	3.82	5.03 b	6.28 b	6.54 b
E	2.28	2.78	3.66	4.23 bc	5.51 bcd	5.44 c
F	2.55	2.92	3.63	4.01 cd	4.76 de	4.94 cd
G	2.36	3.07	3.61	4.23 bc	5.20 cd	5.29 c
CV	15.91%	17.21%	16.56%	13.30%	12.54%	15.94%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L⁻¹ water) with pruning; C= 2 mL L⁻¹ water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L⁻¹ water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L⁻¹ water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L⁻¹ water) liquid BAP without pruning.

Table 5. Average increase in canopy width of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Canopy Width (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	1.90	1.74	2.29 cd	2.47 c	3.10 c	3.15 c
B	1.61	1.80	2.61 bc	2.85 bc	3.71 b	4.12 b
C	1.93	1.68	3.43 a	3.71 a	4.65 a	5.05 a
D	1.79	1.46	3.06 ab	3.21 ab	3.87 b	4.23 b
E	1.68	1.25	1.70 d	1.85 d	2.99 c	4.00 b
F	1.69	1.32	3.12 ab	3.36 ab	4.11 ab	4.24 b
G	1.80	1.73	2.24 cd	2.54 c	2.80 c	2.84 c
CV	15.94%	17.73%	17.41%	11.95%	10.5%	10.0%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

Table 6. Average increase in fruit clusters per branch of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Fruit Clusters per Branch					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	1.24 b	1.04 b	1.06 b	1.15 b	1.27 b	1.28 b
B	1.91 a	2.16 a	2.37 a	2.49 a	2.50 a	2.53 a
C	2.09 a	2.40 a	2.60 a	2.73 a	2.80 a	2.83 a
D	2.05 a	2.37 a	2.58 a	2.71 a	2.78 a	2.82 a
E	1.31 b	1.13 b	1.16 b	1.19 b	1.28 b	1.29 b
F	1.06 b	0.85 b	0.90 b	0.94 b	0.97 b	0.98 b
G	1.24 b	1.11 b	1.19 b	1.22 b	1.32 b	1.35 b
CV	10.16%	13.17%	12.58%	13.09%	12.59%	12.62%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

Leaf Area. The treatment with 75% coconut water and pruning elicited a significant increase in leaf area compared with other treatments at 8, 10, and 12 WAT (Table 4). The lack of significant effects observed during the early stages following the application of the foliar treatments, as well as the pruning treatment, at 2, 4, and 6 WAT may be attributed to the low growth rate of the lateral branches.

Pruning similarly promotes the growth of lateral branches that act as sources of new leaves, together with a better assimilate supply, i.e., greater concentration, towards these growing areas. Even though the number of leaves might decline with pruning, canopy measurement might increase in the medium term due to the growth of new leaves and lateral branches (Liang et al., 2020). The treatment on 75% coconut water with pruning was observed to be significant in increasing leaf area on coffee plants under the productive phase (Table 4). Coconut water has been found to have macronutrients, micronutrients, minerals, and organic plant growth regulators, with cytokinins known to be the most effective growth regulators (Mardhikasari et al, 2019; Shayanthavi et al, 2025). These components are known to increase plant growth rate by enhancing nutrient acquisition and cell division and expansion. This has also been reported in other plants, for example, cocoa,

ISSN: 1412-4718, eISSN: 2581-138x

where the use of coconut water has been found to increase leaf area and dry matter (Rosniawaty et al., 2022; Irvandi & Nurbaiti, 2017). The increase in leaf area observed following the application of coconut water is also closely related to the presence of cytokines in coconut water, which promote cell enlargement and increased cell division, thereby contributing to the increase in leaf area (Wu et al., 2021).

Canopy Width. The treatment of sugarcane molasses at a concentration of 2 ml L^{-1} combined with pruning had a significant effect on increasing canopy width compared to other treatments from 6 to 12 WAT. In contrast, the BAP treatments at 60 ppm without pruning and the 75% coconut water treatment without pruning did not show significant differences from the control (Table 5). This result suggests that, under the conditions of this study, the use of liquid foliar treatments without pruning was insufficient to induce canopy expansion.

Canopy width in coffee plants is heavily dependent on pruning practices due to the important role that it plays in the distribution of photosynthates within plant tissues. By pruning, it reallocates energy from apical dominance at the main shoot to the lateral parts, promoting horizontal growth and subsequently contributing to canopy expansion. Muzaiyyanah et al. (2020) added that during both vegetative and generative growth phases, photosynthates were translocated and accumulated in target organs in accordance with physiological demand within a plant. Photosynthates represent the most active form as an energy source that is utilized for growth activities. Kumar et al. (2017) also showed that pruning regulates canopy development through modification in metabolite allocation toward vegetative growth.

Sugarcane molasses acts as a nutrient source, comprising macronutrients such as nitrogen (N), phosphorus (P), and potassium (K); micronutrients like zinc (Zn), boron (B), manganese (Mn), copper (Cu), molybdenum (Mo), and cobalt (Co); and organic carbon. Nitrogen serves as a precursor during the biosynthesis of amino acids and proteins, which are essential for cell division and growth, thus impacting canopy expansion. Phosphorus participates in energy transmission and photosynthesis, important processes in plant tissue formation and canopy development. Potassium is responsible for the closing and opening of stomata, affecting photosynthesis and transpiration and, thereby, canopy growth.

Fruit Clusters per Branch. This measure refers to the average fruit clusters that are developed on every lateral branch of the plant. Overall, this parameter pertains to the potential of fruiting development, particularly branching, of the coffee plants. Hence, FPP is also another important aspect of the growth and productivity of coffee plants. In terms of its actual influence, it appears that the 75% coconut water, sugarcane molasses at 2 ml L^{-1}, and BAP at 60 ppm in combination with pruning significantly enhanced the level of increase in the number of fruit clusters from 2 to 12 WAT compared to other treatments (Table 6). For its part, the treatment of coconut water, sugarcane molasses, and BAP without pruning did not show any significant difference from the control.

In addition, the results presented in Table 6 suggest that, between 2 and 12 WAT, all pruning treatments were associated with significantly higher numbers of fruit clusters than those of the control and treatment without pruning. In this sense, there is a need to emphasize the significance of pruning on the development of fruit clusters of Arabica coffee plants.

Arabica coffee plants bear fruit in clusters in the sense that they have an optimal number of effective branches to provide for the plants to flower and subsequently bear fruit. Pruning means removing the tip of the plants to direct resource allocation from growth to flowering and bearing fruit. Therefore, the plants are able to maximize the production of effective branches.

Siahaan et al. (2020) found that the effects of pruning, shading, and fertilizers interacted significantly for the purpose of influencing the parameters of coffee plant growth and the formation of cluster fruit. This study emphasized the importance of adding positive cultivation techniques for the productivity of coffee plants, especially the shaded plants.

In the same way, Karim et al. (2021) noted that because of the increased process of pruning in the cultivation of coffee plants under shaded conditions, there was an improvement in the production of fruits in arabica coffee varieties. This encouraged the coffee plants to grow new shoots, which eventually turned into successful branches, making them produce flowers and fruits. It is in this aspect that the process of pruning new shoots is important in the production of fruits in coffee plants.

In addition to pruning, the application of BAP, coconut water, and sugarcane molasses may have contributed to supporting fruit cluster development. BAP, as a cytokinin, is known to promote cell division and stimulate the growth of lateral buds, which can develop into productive branches (Maxiselly et al, 2025). Similarly, coconut water contains natural plant growth regulators such as cytokinins and auxins that may enhance vegetative and reproductive processes (Sosnowski et al., 2025). Meanwhile, sugarcane molasses provides essential nutrients that support metabolic activity and assimilate production (Ali et al., 2019). However, the application of these foliar treatments without pruning did not result in significant differences compared to the control, indicating that their effects are likely complementary rather than dominant. These findings suggest that while pruning serves as the primary driver of fruit cluster formation, the addition of BAP, coconut water, and molasses may support physiological processes that contribute to the development of reproductive structures.

Fruit Clusters per Plant. The fruit clusters per plant refer to the average total fruit clusters formed in each individual plant. This parameter reflects the general potential of fruit production and, therefore, it is an important indicator for the evaluation of the success of the generative growth phase in coffee plants. Statistical analysis showed that at a 95% confidence level, the treatments of 75% coconut water, sugarcane molasses at 2 ml L^{-1}, and BAP at 60 ppm combined with pruning were significantly different from the controls and the respective treatments without pruning within the period of 2-12 WAT (Table 7). The obtained data showed highly significant differences throughout the observation period. On the other hand, treatments without pruning, using the same materials, did not differ significantly from the controls during the same period.

Table 7. Average increase in fruit clusters per plant of productive arabica coffee from 2 to 12 WAT in response to pruning and various liquid foliar treatments

Treatments	Increase in Fruit Clusters per Plant					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	7.25 c	7.41 c	7.44 c	7.95 c	7.95 c	9.30 c
B	10.31 ab	11.51 ab	12.24 ab	12.52 ab	12.52 ab	13.42 ab
C	11.60 a	12.12 ab	12.46 ab	13.19 a	13.19 a	14.64 a
D	11.60 a	12.42 a	13.40 a	14.40 a	14.40 a	15.98 a
E	8.89 bc	9.46 bc	9.56 bc	9.91 bc	9.91 bc	10.94 bc
F	8.15 bc	8.27 c	8.43 c	8.88 c	8.88 c	10.27 bc
G	7.95 bc	8.14 c	8.20 c	8.54 c	8.54 c	9.66 c
CV	18.36%	17.96%	18.38%	19.43%	19.43%	19.42%

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

Table 8. Average fruit weight of arabica coffee plants during the productive phase 2 to 12 WAT in response to pruning and different liquid foliar treatments

Treatments	Increase in Fruit Weight (g)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
A	0.00	0.65	0.00	2.20	0.43	0.55
B	0.53	7.02	11.87	2.93	0.49	1.07
C	9.51	34.80	28.94	3.30	2.88	2.37
D	3.03	5.39	4.57	4.28	5.96	4.17
E	0.00	0.00	0.00	0.00	0.00	0.00
F	0.00	1.65	1.38	0.00	0.00	0.00
G	0.00	0.48	0.78	0.00	0.00	0.35

Note: WAT = Week after treatments. Means followed by different letters within the same column were significantly different based on Duncan multiple range test at the 5% level. Treatments were A= control without pruning and foliar treatments; B= 75% of coconut water (750 mL L^{-1} water) with pruning; C= 2 mL L^{-1} water sugarcane molasses with pruning; D= 60 ppm (0.06 mL L^{-1} water) liquid BAP with pruning; E= 75% of coconut water without pruning; F= 2 mL L^{-1} water sugarcane molasses without pruning; G= 60 ppm (0.06 mL L^{-1} water) liquid BAP without pruning.

The findings show that pruning significantly increases coffee plant productivity. In this case, treatments with coconut water, sugarcane molasses, and BAP, with accompanying pruning, produced more fruit clusters than the control and non-pruned groups. This further proves a point that the interaction of the two processes, this being the supply of yet another nutrient as well as pruning, is essential for the enhancement of plant productivity.

The application of BAP, coconut water, and sugarcane molasses may support this process by enhancing physiological functions. BAP may stimulate the formation of lateral branches, coconut water provides natural growth regulators, and molasses supplies essential nutrients that support assimilate production (Ali et al., 2019; Maxiselly et al, 2025; Sosnowski et al., 2025). However, their application without pruning did not significantly affect fruit cluster formation, indicating that their role is supportive rather than dominant in determining whole-plant productivity.

In arabica coffee, pruning of the shoot is important in enhancing the growth of fruitful branches, which bear flowers and fruits. During the fruiting stage, pruning also plays a facilitative role in ensuring plant nutrients are directed from the vegetative to the fruiting tissues. In this regard, Siahaan et al. (2020) reported that the application of proper pruning practices, alongside proper treatments and homogeneous shade conditions, was important in inducing fruit clusters. The interaction consequently enhanced fruitful branch development and increased coffee production.

In this line, Karim et al. (2021) also emphasized how pruning has helped increase fruiting in the arabica coffee plant. Pruning is very effective in bringing about new shoots, which soon develop into branches in the coffee plants, thus allowing for increased fruit and flower development. With the efficient use of its resources in coffee, the fruits now develop in a better way, allowing arabica coffee to achieve maximum production.

Fruit Weight. According to the data presented, none of the treatments caused a significant impact on the mass of arabica coffee fruits at 2-12 WAT. Although no significant differences were observed, the treatment with sugarcane molasses at 2 ml L^{-1}, combined with pruning, had the highest average fruit weight from 2 to 6 WAT (Table 8). In contrast, from 8 to 12 WAT, the highest average was recorded by the treatment in which BAP in concentrations of 60 ppm was used in combination with pruning.

Therefore, in general, it was seen that the data provided in Table 8 did not show significant effects of the application of pruning and liquid foliar treatments on coffee fruit weight. The condition is closely linked to the biological features of coffee as a perennial species. This means that in a coffee plant, there may be flowers, immature coffee fruits, semi-mature coffee fruits, and mature coffee fruits concurrently, with development at different stages and of different weights (López et al., 2021).

These differences in fruit developmental stages have important implications for resource distribution within the plant. However, it is not possible to homogeneously distribute water, nutrients, and photosynthates to all the fruits. Additionally, the fruits that develop early will enjoy better conditions for the supply of assimilates and nutrients, leading to optimum growth. On the contrary, fruits that develop later will not enjoy the best resources, impacting their weight and quality to some extent. This may be considered as the major factor for the potential effect of pruning and foliar application on coffee fruit weight, as indicated in the study by León-Burgos et al. (2024).

Previous studies have also indicated that in perennial crops, physiological factors are of more dominant importance compared to agronomic practices. This is due to the fact that the biological activities of plants, such as flowering and fruiting, are controlled by internal factors like genetics and environmental factors, which cannot be controlled in the short term. Studies on cocoa (Goudsmit et al., 2023) and tea (Zhang et al., 2022) crops revealed that flowering and fruiting were more important in determining the yield compared to any other factor.

Conclusion

Pruning treatment was identified as a significant treatment that increased all the growth parameters of the arabica coffee plants, namely the number of lateral branches, the length of lateral branches, leaf area, the width of the plant, the number of clusters of fruits per branch, and the number of clusters of fruits per plant. The findings of the study indicated that the application of pruning treatment in addition to the use of sugarcane molasses at a concentration of 2 mL L⁻¹ improved the formation of lateral branches, leaf area, and the number of clusters of fruits per tree. The use of pruning, foliar fertilizer (coconut water and sugarcane molasses), and BAP was effective in supporting the optimal growth of coffee plants during the productive phase.

References

Ali S, Basit A, Khattak A, Shah S, Ullah I, Khan N, Ahmad I, Rauf K, Khan S, Ullah I, Ahmad I. 2021. Managing the growth and flower production of Zinnia (Zinnia elegans) through benzyl amino purine (BAP) application and pinching. Pakistan Journal of Agricultural Research, 34: 29–40.

Ali SE, El Gedaily RA, Mocan A, Farag MA, El-Seedi HR. 2019. Profiling metabolites and biological activities of sugarcane (*Saccharum officinarum* Linn.) juice and its product molasses via a multiplex metabolomics approach. Molecules, 24: 934.

Arsic M, Persson D, Schjoerring J, Thygesen L, Lombi E, Doolette C, Husted S. 2022. Foliar-applied manganese and phosphorus in deficient barley: linking absorption pathways and leaf nutrient status. Physiologia Plantarum, 174: e13761.

Asare G, Bhatt P, Avornyo V, Gyamfi R. 2023. An overview of foliar application of macro and micronutrients on the yield of maize in Ghana. Archives of Agriculture and Environmental Science, 8: 402–426.

Barłóg P, Grzebisz W, Łukowiak R. 2022. Fertilizers and fertilization strategies mitigating soil factors constraining efficiency of nitrogen in plant production. Plants, 11: 1855.

Busato C, Reis EFD, Oliveira MG, Garcia GDO, Busato CCM, Partelli FL. 2022. Different nitrogen levels on vegetative growth and yield of conilon coffee (Coffea canephora). Ciência Rural, 52: e20200770.

Colodetti TV, Rodrigues WN, Brinate SVB, Martins LD, Cavatte PC, Tomaz MA. 2020. The management of orthotropic stems modulates the photosynthetic performance and biomass allocation of productive plants of Arabica coffee. Revista Ceres, 67: 454–463.

Damayanti NLPS, Udayana IGB, Situmeang YP. 2022. Arabica coffee plant response to atonic concentration and production pruning. Sustainable Environment Agricultural Science, 6: 10–15.

Dong T, Duan B, Korpelainen H, Niinemets Ü, Li C. 2019. Asymmetric pruning reveals how organ connectivity alters the functional balance between leaves and roots of Chinese fir. Journal of Experimental Botany, 70: 1941–1953.

Gokavi N, Mote K, Jayakumar M, Raghuramulu Y, Surendran U. 2021. The effect of modified pruning and planting systems on growth, yield, labour use efficiency and economics of Arabica coffee. Scientia Horticulturae, 276: 109764.

Goudsmit E, Rozendaal DM, Tosto A, Slingerland M. 2023. Effects of fertilizer application on cacao pod development, pod nutrient content and yield. Scientia Horticulturae, 313: 111869.

He ZS, Tang R, Li MJ, Jin MR, Xin C, Liu JF, Hong W. 2020. Response of photosynthesis and chlorophyll fluorescence parameters of *Castanopsis kawakamii* seedlings to forest gaps. Forests, 11: 21.

Henningsen JN, Görlach BM, Fernández V, Dölger JL, Buhk A, Mühling KH. 2022. Foliar P application cannot fully restore photosynthetic capacity, P nutrient status, and growth of P deficient maize (*Zea mays* L.). Plants, 11: 2986.

Hönig M, Plíhalová L, Husičková A, Nisler J, Doležal K. 2018. Role of cytokinins in senescence, antioxidant defence and photosynthesis. International Journal of Molecular Sciences, 19: 4045.

Igamberdiev AU, Bykova NV. 2022. Mitochondria in photosynthetic cells: Coordinating redox control and energy balance. Plant Physiology, 191: 2104–2119.

Ikeda Y, Zalabák D, Kubalová I, Králová M, Brenner WG, Aida M. 2021. Interpreting cytokinin action as anterograde signaling and beyond. Frontiers in Plant Science, 12: 641257.

Irvandi D, Nurbaiti N. 2017. Pengaruh pupuk NPK dan air kelapa sebagai zat pengatur tumbuh alami terhadap pertumbuhan bibit kakao (Theobroma cacao L.) di medium subsoil.

Jing D, Du Z, Wang M, Wang Q, Liu F, Dong Y. 2018. Regulatory effects of root pruning on leaf nutrients, photosynthesis, and growth of trees in a closed-canopy poplar plantation. PLoS ONE, 13: e0197515.

Karim A, Hifnalisa H, Manfarizah M. 2021. Analysis of Arabica coffee productivity due to shading, pruning, and coffee pulp-husk organic fertilizer treatments.

Kumar P, Karuna K, Mankar A, Tiwari DK, Singh RR. 2017. Influence of pruning severity on plant canopy architecture for yield and quality attributing traits of guava (Psidium guajava L.) cv. Pant Prabhat. Research in Environment and Life Sciences, 10: 560–564.

Kurniawan S, Nugroho RMYAP, Ustiatik R, Nita I, Nugroho GA, Prayogo C, Anderson CWN. 2024. Soil nitrogen dynamics affected by coffee (Coffea arabica) canopy and fertilizer management in coffee-based agroforestry. Agroforestry Systems, 98: 1323–1341.

León-Burgos AF, Sáenz JRR, Quinchua LCI, Toro-Herrera MA, Unigarro CA, Osorio V, Balaguera-López HE. 2024. Increased fruit load influences vegetative growth, dry mass partitioning, and bean quality attributes in full-sun coffee cultivation. Frontiers in Sustainable Food Systems, 8: 1379207.

Liang F, Yang C, Sui L, Xu S, Hesheng Y, Zhang W. 2020. Flumetralin and dimethyl piperidinium chloride alter light distribution in cotton canopies by optimizing the spatial configuration of leaves and bolls. Journal of Integrative Agriculture, 19: 1777–1788.

Little N, DiTommaso A, Westbrook A, Ketterings Q, Mohler C. 2021. Effects of fertility amendments on weed growth and weed–crop competition: a review. Weed Science, 69: 132–146.

López M, Santos I, Oliveira R, Lima A, Cardon C, Chalfun-Júnior A. 2021. An overview of the endogenous and environmental factors related to the Coffea arabica flowering process. Beverage Plant Research.

Ma X, Li F, Chen Y, Chang Y, Lian X, Li Y, Ye L, Yin T, Lu X. 2022. Effects of fertilization approaches on plant development and fertilizer use of citrus. Plants, 11: 2547.

Mao X, Gu J, Wang F, Wang K, Liu R, Hong Y, Wang Y, Han F. 2024. Yield, quality, and nitrogen leaching of open-field tomato in response to different nitrogen application measures in Northwestern China. Plants, 13: 0924.

Mardhikasari S, Yunus A, Samanhudi S. 2019. Modification of media for banana in vitro propagation with foliar fertilizer and coconut water in cv. Rajabulu. Caraka Tani: Journal of Sustainable Agriculture, 35: 45–53.

Maxiselly Y, Samuel J, Suherman C. 2025. The effect of topping and various cytokinin-based PRGs applications on immature Liberica coffee growth. Kultivasi, 24.

Mohammed T, Worku M. 2025. Pruning and fertilising effects on yield and yield components of Arabica coffee in its centre of origin in southwest Ethiopia. The Journal of Agricultural Science, 163: 493–502.

Muliasari A, Dewi R, Rochmah H, Malala A, Adinurani P. 2021. Improvement generative growth of Coffea arabica L. using plant growth regulators and pruning. E3S Web of Conferences, 226: 00003.

Muzaiyyanah S, Kristiono A, Kuntyastuty H. 2020. Pola distribusi fotosintat dan pertumbuhan Wilis dan Tanggamus di tanah optimal dan salin. Prosiding SEMNAS Pertanian 2020: 106–118.

Niu J, Liu C, Huang M, Liu K, Yan D. 2020. Effects of foliar fertilization: a review of current status and future perspectives. Journal of Soil Science and Plant Nutrition, 21: 104–118.

Rahman M, Malek M, Hossain M, Islam M. 2024. Effect of pruning on growth, yield and quality of coffee. East African Scholars Journal of Agriculture and Life Sciences, 7: 15–22.

Rico, Darma R, Salman D, Mahyuddin. 2021. Problems identification of Arabica coffee commodities on traditional farming in Indonesia: A review. IOP Conference Series: Earth and Environmental Science, 886: 012069.

Rodrigues WN, Martins LD, Apostólico MA, Colodetti TV, Brinate SVB, Christo BF, Tomaz MA. 2017. Coffee pruning: importance of diversity among genotypes of Coffea arabica. African Journal of Agricultural Research.

Rohani RTS, Prayogo C, Suprayogo D, Wicaksono KS. 2024. The effect of coffee canopy pruning and fertilization on coffee growth and soil physical properties. Journal of Applied Agricultural Science and Technology, 8(1): 29–49.

Rosniawaty S, Suherman C, Ariyanti M, Sudirja R, Situmorang ES. 2022. Aplikasi pupuk organik cair dan air kelapa terhadap pertumbuhan tanaman kopi Arabika (Coffea arabica L.). Prosiding Seminar Nasional Pertanian Pesisir, 1: 103–110.

Rowe RL, Prayogo C, Oakley S, Hairiah K, van Noordwijk M, Wicaksono KP, Kurniawan S, Fitch A, Cahyono ED, Suprayogo D, McNamara NP. 2022. Improved coffee management by farmers in state forest plantations in Indonesia: An experimental platform. Land, 11: 671.

Salman A, Abdulrasool I. 2022. Effect of ozone enrichment and spraying with coconut water and moringa extract on vegetative growth and yield of broccoli plant under hydroponic system with modified NFT technology. Iraqi Journal of Agricultural Sciences, 53: 349–360.

Sharma D, Thokchom R, Thakur K. 2025. Pruning and nitrogen fertilization interactions on growth and productivity of New Castle apricot (*Prunus armeniaca* L.) trees. Journal of Animal & Plant Sciences, 35(2): 341–353.

Shayanthavi S, Kapilan R, Wickramasinghe I. 2025. A comprehensive review of coconut liquid endosperm (Cocos nucifera L.): Composition, physicochemical characteristics, antimicrobial and antioxidant properties, and applications in microbial and tissue culture media. Journal of Science of the University of Kelaniya, 18: 1–18.

Siahaan ASA, Masrul HE, Chairani H, Karim K. 2020. The growth and yield of Arabica coffee in shade conditions under different pruning and fertilizing treatments. Russian Journal of Agricultural and Socio-Economic Sciences, 99: 40–46.

Soares D, da Silva E, de Figueiredo Carvalho M, Pereira F, Guimarães R. 2022. Leaf anatomy, physiology and vegetative growth of fertigated Coffea arabica L. trees after exposure to pruning. Coffee Science, 16: e161962.

Sosnowski J, Truba M, Vasileva V. 2023. The impact of auxin and cytokinin on the growth and development of selected crops. Agriculture, 13: 724.

Stephen G, Shitindi M, Bura M, Kahangwa C, Nassary E. 2024. Harnessing the potential of sugarcane-based liquid byproducts—molasses and spentwash (vinasse) for enhanced soil health and environmental quality: a systematic review. Frontiers in Agronomy, 6: 1358076.

United States Department of Agriculture. 2024. Coffee: World Markets and Trade. Global Market Analysis, Foreign Agricultural Service, USDA.

Vinecky F, Davrieux F, Mera AC, Alves GSC, Lavagnini GV, Leroy T, Bonnot F, Rocha OC, Bartholo GF, Guerra AF, Rodrigues GC, Marraccini P, Andrade AC. 2017. Controlled irrigation and nitrogen, phosphorous and potassium fertilization affect the biochemical composition and quality of Arabica coffee beans. The Journal of Agricultural Science.

Wu W, Du K, Kang X, Wei H. 2021. The diverse roles of cytokinins in regulating leaf development. Horticulture Research, 8: 118.

Xie S, Li D, Liu Z, Wang Y, Ren Z, Li C, Hu D. 2023. Foliar fertilizer application alters the effect of girdling on nutrient contents and yield of Camellia oleifera. Life, 13: 51.

Yang J, Xie S, Du D, Wei H, Zhou W, Zhang Y, Tang Z, Li D, Liu Y. 2023. Effect of pruning treatment on growth characteristics and metabolites in Eucommia ulmoides Oliver (E. ulmoides). Forests, 14: 2439.

Zarea M, Karimi N. 2023. Grain yield and quality of wheat are improved through post-flowering foliar application of zinc and 6-benzylaminopurine under water deficit condition. Frontiers in Plant Science, 13: 1068649.

Zhang C, Yan K, Lin L, Fang Y, Zhang X. 2022. Effects of source–sink alteration by pruning on physiological parameters and fruit production of Rosa roxburghii Tratt. on the Yunnan–Guizhou Plateau in China. Photosynthetica, 60: 190–199.

Zhang Q, Zhang Y, Wang Y, Zou J, Lin S, Chen M, Miao P, Jia X, Cheng P, Pang X, Ye J, Wang H. 2022. Transcriptomic analysis of the effect of pruning on growth, quality, and yield of Wuyi Rock tea. Plants, 12: 3625.

Zhong L, Xu S, Xu S, Zhou W, Lu Z, Jin B, Wang L. 2024. Consecutive pruning enhances leaf flavonoids, leaf yield, and cutting rooting in Ginkgo biloba. Forests, 15: 761.

Jurnal Kultivasi Vol. 25 (1) April 2026
ISSN: 1412-4718, eISSN: 2581-138x

Kurniawan RR · Ahadiyat YR · Tini EW

Growth of vanilla cuttings under modified acid soil media and application of several plant growth regulators

Abstract. This research aimed to optimize the growth of vanilla (*Vanilla planifolia*) cuttings in acid soils by modifying the growing media and applying several types of plant growth regulators (PGRs). Several types of PGRs, including synthetic compounds such as indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP), as well as natural extracts from shallot (*Allium ascalonicum* L.) and moringa leaves (*Moringa oleifera*), were tested to enhance plant performance in acid-growing conditions. The primary objectives were to determine the optimal soil amendment formula and the most effective PGR type for promoting vanilla growth, and to develop a measurable, easy-to-use PGR formulation accessible to farmers. The study was conducted experimentally using a randomized complete block design (RCBD) with the first factor being modified acid soil media and the second factor being the type of PGRs. The results showed that both 100 ppm BAP (Z2) and 10% shallot extract (Z3) significantly outperformed other PGR treatments across all parameters and observation times. For instance, Z2 produced the highest recorded values for internodes (15.11 internodes) at 90 DAP, number of leaves (10.26 leaves) at 60 DAP, total leaf area (413.74 cm^2) at 90 DAP, and aerial roots (12.22 roots) at 90 DAP. Conversely, Z3 demonstrated superior performance in the number of leaves (15.56 leaves) at 90 DAP and total leaf area (421.95 cm^2) at 90 DAP. This treatment can be recommended as an effective strategy for enhancing the establishment of vanilla cuttings in acidic soil conditions.

Keywords: 6-benzylaminopurine · Indole-3-acetic acid · PGRs · Shallot · *Vanilla planifolia*.

Submitted: 24 February 2026, Accepted: 27 April 2026, Published: 30 April 2026
DOI: https://doi.org/10.24198/kultivasi.v25i1.69953

Kurniawan RR[1] · Ahadiyat YR[1] · Tini EW[*1]
[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Jl. DR. Soeparno No.63, Purwokerto 53122, Central Java, Indonesia
Correspondence: etik.tini@unsoed.ac.id

Kurniawan RR, Ahadiyat YR, Tini EW. 2026. Growth of vanilla cuttings under modified acid soil media and application of several plant growth regulators. Jurnal Kultivasi, 25 (1): 100-115.

Introduction

Vanilla (*Vanilla planifolia*) is a high-value plantation commodity in both local and export markets. Its high economic value has led to vanilla being called "green gold." Indonesia is the second-largest producer of vanilla in the global market. According to data from World Population Review (2024), the three largest vanilla-producing countries in 2023 were Madagascar (3.11 thousand tons), Indonesia (1.83 thousand tons), and Mexico (508 tons). Indonesia's production decreased from 1.97 thousand tons in 2022. Indonesian grade A vanilla is highly valued, with a price range of USD140-200/kg, exceeding the global market average price of EUR175.56/kg for whole vanilla and EUR270.40/kg for vanilla extract in 2022 (Munarso et al., 2024). Indonesian vanilla production from 2015 to 2024 (in terms of dried pods) has fluctuated, but showed a downward trend. Production in 2015 reached 1.74 thousand tons and was projected to decline to 1.56 thousand tons in 2024, with an average annual growth rate of -1.25% (Pertanian Sekretariat Jenderal Kementrian, 2024)

There is a need to increase the supply of vanilla planting materials and to ensure the availability of healthy seedlings. The availability of healthy planting materials is one of the requirements for successful large-scale vanilla cultivation (Murthy et al., 2010). Propagating vanilla by seed is less efficient because germination is slow, resulting in inconsistent plants. Therefore, stem cutting is more commonly used. This method accelerates plant production. Its advantages include producing plants with characteristics identical to those of the parent plant and lower costs (Ayyubi et al., 2019).Although the demand for vanilla is high, production remains limited due to farmers' challenges in managing agronomic conditions and the scarcity of fertile land.

It is necessary to explore marginal acidic land, one of the types of marginal land commonly found in Indonesia (99.56 million ha) (Hidayat & Mulyani, 2002). Acid soils are characterized by infertility, with a low pH of <5.5. High acidity levels result from the weathering of sulphide minerals, the leaching of base cations (Ca^{2+}, Mg^{2+}, K^{+}), and the decomposition of organic matter, which releases H^{+} (Brady & Weil, 2017; Food and Agriculture Organization of the United Nations, 2020). Vanilla grows optimally in a medium with a neutral pH of 6.5-7 (Divakaran et al., 2024; Havkin-Frenkel & Belanger, 2018). Acidic soils cause nutrient deficiencies in plants due to the binding and leaching of nutrients. High Al^{3+} ratios (>60%) interfere with Ca^{2+} and Mg^{2+} absorption (Food and Agriculture Organization of the United Nations, 2020). Al^{3+} inhibits root elongation, reducing nutrient and water absorption (Kochian et al., 2015). As a result, plants experience physiological stress, affecting their growth response.

At low pH (<5), plants have reduced activity of auxin-synthesizing enzymes, which are essential for cell differentiation (Taiz et al., 2015). The main method of vanilla propagation is through cuttings, but the roots of cuttings are susceptible to stress in acidic soils. Vanilla cuttings rely on adventitious root growth, which is sensitive to Al^{3+}. Exposure to Al^{3+} not only reduces root respiration and disrupts the uptake and transport of Ca^{2+}, Mg^{2+}, and H_2O, but also, upon root absorption, induces the production of reactive oxygen species (ROS), including the superoxide anion (O_2^{-}), which subsequently damages biomolecules such as lipids and nucleic acids (Berndt, 2024). Cuttings experience stress in acidic soils, resulting in plants failing to reach physiological maturity in the vegetative phase, with low biomass and limited carbohydrate reserves.

This study was based on the urgent need to optimize acidic land as a strategy for utilizing marginal land in vanilla cultivation. Acidic soil media were modified by incorporating organic matter and dolomite. Organic matter helps reduce Al toxicity and Fe2+, increase soil pH, and improve phosphorus availability in acidic soils (Suyanto, 2023). The application of dolomite increases the pH of acidic soil, with fine-textured dolomite showing higher effectiveness in neutralizing soil acidity (Wu et al., 2021). The application of exogenous PGRs and phytohormones is used to enhance the growth of vanilla cuttings in acidic soils. Shallot extract at 300 g/L (P_1) consistently produced the highest vegetative growth in vanilla cuttings under both elicitor treatments, namely *Coleus scutellarioides* extract (E1) and *Amaranthus spinosus* extract (E_2), with the respective values for P_1E_1 and P_1E_2 as follows: shoot length (44.97 cm and 46.60 cm), leaf number (7.67 and 7.33), leaf length (8.25 cm and 7.43 cm), leaf width (2.71 cm and 2.70 cm), and stem diameter (0.79 cm and 0.87

ISSN: 1412-4718, eISSN: 2581-138x

cm), compared to banana weevil extract (P_2), bamboo shoot extract (P_3), coconut water (P_4), and sprouts extract (P_5) (Rampe et al., 2024).

Moringa leaf extract as a biostimulant consistently improved the growth of black orchid (*Coelogyne pandurata*) plantlets across all growing media, increasing sapling height (up to 3.66 cm compared to 2.82 cm in the control on wood sawdust), leaf number (up to 4.80 compared to 3.40 on cocopeat), leaf length (up to 2.48 cm compared to 2.13 cm on coconut coir), leaf width (up to 0.64 cm compared to 0.53 cm on cocopeat), root number (up to 7.60 compared to 4.80 on cocopeat), and root length (up to 1.80 cm compared to 0.91 cm on sugarcane waste) (Zakiah & Turnip, 2023). In the orchid tissue culture study by Rathnayaka et al. (2023), moringa leaf extract at ¼ KnC + 15 ml/l produced the highest number of leaves (5.2 per plantlet) and roots (6 per plantlet), while at full KnC + 5 ml/l it yielded the highest number of shoots (3.5 per plantlet) (Unit & Garden, 2023).

Aluminium stress increases auxin accumulation in the root apical meristem and transition zone but decreases it in the elongation zone, whereas exogenous IAA partially restores auxin distribution, reduces Al accumulation in the cell wall, and alleviates toxicity (Li et al., 2018; Tao et al., 2023). Exogenous IAA application in the elongation zone can partially restore this inhibition by minimizing the disruption of auxin transport under Al toxicity. Exogenous IAA alleviates aluminium toxicity by reducing Al accumulation in the cell wall of alfalfa, whereas in tea plants, moderate Al supply promotes root growth through IAA accumulation, indicating that optimal IAA levels support root development under Al exposure (Gao et al., 2022; Wang et al., 2016). Modification of the growing medium and application of PGRs were done to stimulate the physiological performance and growth of vanilla plants in acidic soils.

Materials and Methods

Plant material. The research was conducted in Karangbanjar Village, Bojongsari District, Purbalingga (7°21'00"S 109°20'18" E) at an altitude of 148 meters above sea level. The research was conducted from November 2025 to February 2026. The vanilla cultivar used in this study was Vania 1. The cuttings had three nodes and two to three leaves. They were planted in a planter bag as a growing medium at a depth of 10 cm. One month after planting, the cuttings were tied to 130-cm-tall climbing poles made of coco fiber to support climbing. The average environmental conditions were 26.5°C, 83.63% humidity, 4.16 mm rainfall, and 15,957 lux light intensity.

The extraction of shallot and moringa was carried out as follows. Fresh shallot bulbs and fresh moringa leaves were separately washed, cut into small pieces, and then homogenized using an electric blender. For each extract, 100 g of plant material was blended with 1 L of distilled water (10%, w/v) at room temperature (24–26 ± 2°C) for 5 minutes until a homogeneous slurry was obtained. The resulting mixture was filtered twice through cheesecloth (pore size ± 0.5 mm) to remove coarse debris, and the clear filtrate was collected. The filtrate was stored in sterile amber glass bottles and used within 24 hours of preparation to prevent degradation of bioactive compounds.

Experimental design and treatments. The experiment was conducted under 75% shading net. The planter bags had a capacity of 35 L, and they were spaced 35 × 35 cm apart on acidic soil with a pH of 4.947. This study employed a factorial randomized complete block design (RCBD) with two factors: modified acidic soil media (three levels) and type of plant growth regulators (five levels), resulting in 15 treatment combinations. The experiment was arranged in three blocks as replicates, yielding 45 experimental units. Each experimental unit consisted of three planter bags (subsamples), with one plant per bag, giving a total of 135 plants. Data from the three subsamples within each experimental unit were averaged prior to statistical analysis. The first factor was modified acid soil media: acid soil (S_1), acid soil + 36 g of dolomite (S_2), and acid soil + 250 g of goat manure fertilizer + 36 g of dolomite (S_3). The second factor was the type of PGRs: control without PGRs (Z_0), 100 ppm IAA (Z_1), 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4). The observation variables included tendrils length, number of internodes, number of leaves, total leaf area, and number of aerial roots. Observation data were statistically analyzed using analysis of variance (ANOVA) at the 5% significance level to determine treatment effects. When a significant effect was detected, Duncan's Multiple Range Test (DMRT) at the 5% level and regression analysis were conducted for further evaluation.

Tendrils length (cm). Tendrils length was measured using a tape measure from the base of the stem at ground level to the tip of the longest tendril (growing point). Measurements were taken at 60 days after planting (DAP) and at the end of the study, at 90 DAP.

Number of internodes. The number of internodes was measured by counting all fully formed nodes along the main stem of the plant. Measurements were taken at 30, 60, and 90 DAP.

Number of leaves. The number of leaves was recorded by counting all fully expanded, healthy leaves per plant. Leaf number was measured at 30, 60, and 90 DAP.

Total leaf area (cm²). Total leaf area was measured first by assessing the sample leaf area using a non-destructive computer vision method through the Petiole Pro application (version 24.10.01) on a smartphone (Figure 1). A calibration sheet containing eight markers was printed from the application at 100% scale and used as a reference. Small leaves were placed on the calibration sheet, whereas large leaves were positioned next to it on a contrasting surface, ensuring that the entire leaf and all eight markers were captured within a single frame. Images were taken under uniform lighting to avoid shadows. The application then automatically processed the image and displayed the leaf area per leaf (cm²).

Total leaf area was calculated according to Campbell and Norman (1998), as follows.

$$\text{Total leaf area} = \left(\frac{\textit{total leaf area of sampled leaves}}{\textit{Number of leaf samples}}\right) \text{x total leaves per plant}$$

Number of aerial roots. The number of aerial roots was counted on all visible roots emerging from each stem segment above the surface of the growing medium. Counts were recorded at 30, 60, and 90 DAP.

Results and Discussion

Before discussing the growth responses of vanilla cuttings, it is essential to first describe the changes in soil properties resulting from the applied treatments, as soil quality is a key determinant of rooting success in acidic soils. In this study, the initial acidic soil was characterized by low pH (<5.0), high exchangeable Al^{3+}, and very limited phosphorus availability. The application of organic matter and dolomite significantly improved soil chemical properties, including increasing soil pH to near-neutral levels, reducing soluble Al^{3+} and Fe^{2+} concentrations, and enhancing available phosphorus, although the magnitude of improvement varied among treatment combinations. Whether these improvements in soil quality significantly affected vanilla growth or not should be discussed, as they may determine the physiological response of cuttings to the modified root-zone environment.

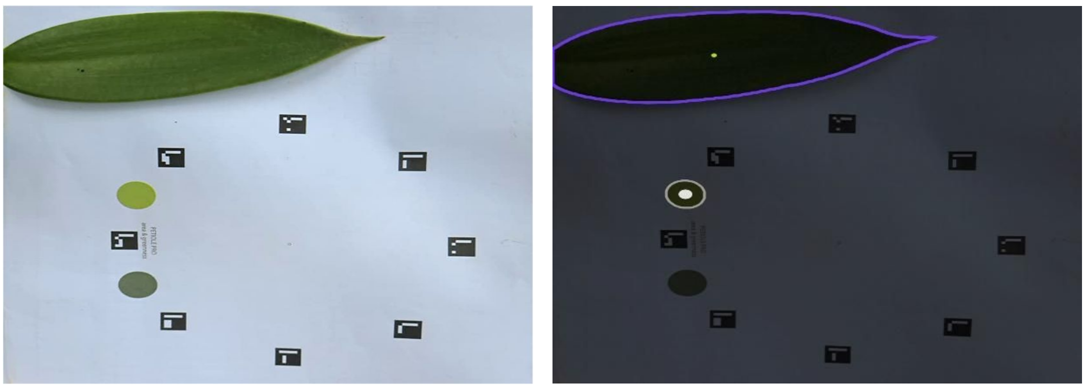


Figure 1. Assessing the sample leaf area using a non-destructive computer vision method through the Petiole Pro application (version 24.10.01)

Table 1. The effect of modified acid soil media and type of plant growth regulator on tendrils length

Treatments	Tendrils Length at 60 DAP (cm)	Tendrils Length at 90 DAP (cm)
Modified acid soil media (S)		
S_1 (acid soil)	65.17±13.02a	77.80±18.13a
S_2 (acid soil + 36 g dolomite)	66.40±16.14a	78.93±21.23a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	62.23±12.63a	82.80±9.25a
Type of PGRs (Z)		
Z_0 (Control)	49.56±3.27b	61.11±13.06b
Z_1 (100 ppm IAA)	64.39±16.80ab	84.11±15.54ab
Z_2 (100 ppm BAP)	74.50±6.83a	87.67±8.57a
Z_3 (10% shallot extract)	70.06±13.56ab	86.67±17.58a
Z_4 (10% moringa leaf extract)	64.50±12.13ab	79.67±15.95ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 2. The effect of modified acid soil media and type of plant growth regulator on number of internodes

Treatments	Number of Internodes at 30 DAP	Number of Internodes at 60 DAP	Number of Internodes at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	7.57±1.12a	8.98±1.29a	14.00±2.01a
S_2 (acid soil + 36 g dolomite)	8.53±1.38a	10.28±1.38a	13.93±2.13a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	8.17±1.13a	9.52±1.20a	13.67±1.65a
Type of PGRs (Z)			
Z_0 (Control)	6.46±0.45b	8.22±0.40b	12.11±1.02b
Z_1 (100 ppm IAA)	8.30±1.57ab	9.39±2.02ab	13.22±2.22ab
Z_2 (100 ppm BAP)	8.85±0.62a	10.70±0.94a	15.11±1.07a
Z_3 (10% shallot extract)	8.56±0.51a	10.00±0.73ab	14.78±2.22a
Z_4 (10% moringa leaf extract)	8.28±1.22ab	9.65±1.20ab	14.11±1.35ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 3. The effect of combined treatments of modified acid soil media and type of plant growth regulator on number of internodes

Treatments	Number of Internodes at 30 DAP	Number of Internodes at 60 DAP	Number of Internodes at 90 DAP
Modified acid soil media (S) x Type of PGRs (Z)			
S_1Z_0	6.00±1.73a	7.78±2.02a	12.33±1.53bc
S_1Z_1	7.44±2.00a	8.11±2.17a	12.67±2.52bc
S_1Z_2	8.17±0.76a	9.78±0.48a	14.33±2.52abc
S_1Z_3	9.00±0.50a	10.83±1.04a	17.33±1.53a
S_1Z_4	7.22±0.69a	8.39±1.34a	13.33±3.51abc
S_2Z_0	6.50±1.80a	8.56±0.36a	11.00±1.00c
S_2Z_1	10.11±1.64a	11.72±0.75a	15.67±0.58ab
S_2Z_2	9.39±1.51a	11.67±1.53a	16.33±2.08ab
S_2Z_3	8.67±2.33a	9.67±2.60a	13.33±2.08abc
S_2Z_4	8.00±2.08a	9.78±2.83a	13.33±2.08abc
S_3Z_0	6.89±1.26a	8.33±0.58a	13.00±1.00bc
S_3Z_1	7.33±1.20a	8.33±2.08a	11.33±2.89c
S_3Z_2	9.00±0.67a	10.67±0.88a	14.67±1.53abc
S_3Z_3	8.00±0.00a	9.50±0.87a	13.67±0.58abc
S_3Z_4	9.61±0.10a	10.78±1.07a	15.67±0.58ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$

Based on Table 1, the type of PGRs influenced the tendrils length growth parameter. At 60 DAP, the 100 ppm BAP treatment (Z_2) produced the highest mean tendrils length (74.50 cm), which was 50.32% greater than the control (Z_0; 49.56 cm). The highest mean tendrils lengths at 90 DAP were 87.67 cm and 86.67 cm in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, respectively. Modified acidic soil medium had no statistically significant effect on tendrils length of vanilla cuttings, where the treatment mean square (68.72) was substantially smaller than the residual mean square (255.90). This indicates that variation among soil treatments was negligible relative to random experimental error, leading to failure to reject the null hypothesis. The interaction between soil medium modification and PGR type was also not significant for tendrils length.

The type of PGR treatment affected the number of internodes (Figure 2A and 3). Based on Table 2, at 30 DAP, the highest mean number of internodes was observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with values of 8.85 and 8.56 internodes, respectively. These values were 37.00% and 32.51% higher than the control (Z_0), which had 6.46 internodes. The highest number of internodes at 60 DAP was recorded in the 100 ppm BAP treatment (Z_2), with 10.70 internodes, which was 30.17% higher than the control (Z_0), which had 8.22 internodes. At 90 DAP, the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments produced the highest numbers of internodes, with 15.11 and 14.78 internodes, respectively, representing increases of 24.77% and 22.05% over the control (Z_0).

The modified acidic soil medium had no effect on the number of internodes in this study. Modified acidic soil medium did not have a statistically significant effect on the number of internodes at both 60 DAP ($p = 0.100$) and 90 DAP ($p = 0.887$). At 60 DAP, the p-value approached but did not reach the 0.05 significance threshold, while at 90 DAP, the effect was clearly negligible. The very low F-value at 90 DAP (0.120) indicates that variation among soil treatments was far smaller than residual error (MS treatment = 0.467, MS residual = 3.890). Consequently, we fail to reject the null hypothesis, meaning that under the conditions tested, modifying the acidic soil did not lead to measurable changes in internode formation. However, the interaction between the modified acidic soil medium and the type of PGR significantly affected the number of internodes at 90 DAP. The highest number of internodes at 90 DAP was observed in the acidic soil plus 10% shallot extract treatment (S_1Z_3), with 17.33 internodes, representing a 40.55% increase over the control (S_1Z_0), which had 12.33 internodes (Table 3).

Table 4 indicate that the type of PGR significantly influenced the number of leaves. At 30 DAP, the highest mean leaf counts were observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with 8.52 and 8.80 leaves, respectively. These values represented increases of 32.92% and 37.29%, respectively, compared with the control (Z_0). At 60 DAP, the 100 ppm BAP treatment (Z_2) produced the highest leaf count, with 10.26 leaves, representing a 25.27% increase over the control (Z_0). At 90 DAP, the 10% shallot extract treatment (Z_3) produced the highest leaf count, with 15.56 leaves, representing a 27.33% increase over the control (Z_0). The number of leaves was not affected by the modified acidic soil medium. In addition, the interaction between the modified acidic soil medium and PGR type had no significant effect on leaf number. Modified acidic soil medium did not significantly affect leaf number at any observation time (30 DAP: $p = 0.536$; 60 DAP: $p = 0.070$; 90 DAP: $p = 0.357$). Similarly, the interaction between modified acidic soil medium and PGR type was not significant across all time points (30 DAP: $p = 0.856$; 60 DAP: $p = 0.167$; 90 DAP: $p = 0.114$). These results indicate that variation in leaf number was primarily explained by PGR type, which showed a significant effect at all observation times ($p < 0.05$), whereas soil modification and its interaction with PGRs contributed negligibly under the tested conditions.

ISSN: 1412-4718, eISSN: 2581-138x

Table 4. The effect of modified acid soil media and type of plant growth regulator on number of leaves

Treatments	Number of Leaves at 30 DAP	Number of Leaves at 60 DAP	Number of Leaves at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	7.56±1.09a	8.91±1.04a	13.60±2.01a
S_2 (acid soil + 36 g dolomite)	8.16±1.16a	10.17±1.38a	14.73±2.13a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	8.07±0.92a	9.63±1.09a	14.40±1.55a
Type of PGRs (Z)			
Z_0 (Control)	6.41±0.43b	8.19±0.54b	12.22±0.84b
Z_1 (100 ppm IAA)	7.87±0.84ab	9.65±1.70ab	13.78±2.52ab
Z_2 (100 ppm BAP)	8.52±0.45a	10.26±0.72a	15.11±1.35ab
Z_3 (10% shallot extract)	8.80±0.31a	10.06±0.63ab	15.56±1.26a
Z_4 (10% moringa leaf extract)	8.04ab±0.97ab	9.70±1.50ab	14.56±1.68ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Table 5. The effect of modified acid soil media and type of plant growth regulator on total leaf area

Treatments	Total Leaf Area at 60 DAP (cm²)	Total Leaf Area at 90 DAP (cm²)
Modified acid soil media (S)		
S_1 (acid soil)	260.47±71.26a	270.73±48.55b
S_2 (acid soil + 36 g dolomite)	319.17±82.51a	377.11±85.52a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	338.06±129.84a	403.20±80.10a
Type of PGRs (Z)		
Z_0 (Control)	194.29±23.03b	274.71±71.00b
Z_1 (100 ppm IAA)	301.90±103.92ab	289.23±41.17ab
Z_2 (100 ppm BAP)	341.21±28.27a	413.74±100.32a
Z_3 (10% shallot extract)	328.78±19.41a	421.95±78.90a
Z_4 (10% moringa leaf extract)	363.33±163.88a	352.09±76.57ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Table 6. The effect of combined treatments of modified acid soil media and type of plant growth regulator on total leaf area and leaf greenness

Treatments	Total Leaf Area at 60 DAP (cm²)	Total Leaf Area at 90 DAP (cm²)
Modified acid soil media (S) x Type of PGRs (Z)		
S_1Z_0	173.02±85.19d	207.38±65.91a
S_1Z_1	237.91±60.68bcd	244.46±29.47a
S_1Z_2	319.98±53.26bcd	302.10±56.26a
S_1Z_3	346.30±27.79bcd	331.83±33.46a
S_1Z_4	225.16±136.03cd	267.87±46.55a
S_2Z_0	191.09±82.35cd	267.85±112.79a
S_2Z_1	421.81±88.17ab	325.44±68.54a
S_2Z_2	330.35±83.22bcd	442.79±186.23a
S_2Z_3	332.14±85.74bcd	478.58±187.89a
S_2Z_4	320.44±146.71bcd	370.86±121.51a
S_3Z_0	218.74±66.27cd	348.89±33.01a
S_3Z_1	245.99±61.27bcd	297.80±70.45a
S_3Z_2	373.30±123.82abc	496.32±144.52a
S_3Z_3	307.91±50.90bcd	455.44±158.40a
S_3Z_4	544.39±136.43a	417.52±10.94a

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at p<0.05.

Based on Table 5, the modified acidic soil medium had no effect on total leaf area at 60 DAP. However, it significantly affected total leaf area at 90 DAP. The highest total leaf area at 90 DAP was observed in the acid soil + 36 g dolomite (S_2) and acid soil + 250 g goat manure fertilizer + 36 g dolomite (S_3) treatments, with values of 377.11 and 403.20 cm², respectively, representing increases of 39.29% and 49.93% over the control (Z_0). The type of PGR affected total leaf area. At 60 DAP, the highest mean total leaf area was observed in the 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4) treatments, with values of 341.21, 328.78, and 363.33 cm², respectively. These values were 32.92% and 37.29% greater than those of the control (Z_0; 194.29 cm²). At 90 DAP, the highest total leaf area was recorded in the 10% shallot extract treatment (Z_3), at 421.95 cm², which was 53.60% greater than that of the control (Z_0).

The interaction between the modified acidic soil media and PGR type significantly affected total leaf area at 60 DAP, but not at 90 DAP. Based on Table 6 the highest total leaf area at 60 DAP was recorded in the treatment combining acidic soil, 250 g goat manure fertilizer, 36 g dolomite, and 10% moringa leaf extract (S_3Z_4), with a value of 544.39 cm². This was 214.65% higher than that of the acidic soil treatment without PGRs (S_1Z_0). The significant F-value for the interaction (p = 0.024) confirms that the effect of modified acidic soil medium on total leaf area depends on the type of PGR applied.

Table 7. The effect of modified acid soil media and type of plant growth regulator on number of aerial roots

Treatments	Number of Aerial Roots at 30 DAP	Number of Aerial Roots at 60 DAP	Number of Aerial Roots at 90 DAP
Modified acid soil media (S)			
S_1 (acid soil)	5.04±1.10a	6.49±1.21a	10.53±1.73a
S_2 (acid soil + 36 g dolomite)	5.42±1.64a	7.04±1.43a	11.67±2.82a
S_3 (acid soil + 250 g goat manure fertilizer + 36 g dolomite)	5.18±1.28a	6.36±1.55a	10.87±1.50a
Type of PGRs (Z)			
Z_0 (Control)	3.76±0.52b	4.87±0.45b	8.44±1.17b
Z_1 (100 ppm IAA)	5.37±1.48ab	6.87±1.63ab	11.22±2.34a
Z_2 (100 ppm BAP)	6.06±0.87a	7.22±0.39a	12.11±1.64a
Z_3 (10% shallot extract)	5.70±0.79a	7.37±1.11a	12.56±1.07a
Z_4 (10% moringa leaf extract)	5.19±1.66ab	6.81±1.36ab	10.78±1.39ab

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

Table 8. The effect of combined treatments of modified acid soil media and type of plant growth regulator on number of aerial roots

Treatments	Number of Aerial Roots at 30 DAP	Number of Aerial Roots at 60 DAP	Number of Aerial Roots at 90 DAP
Modified acid soil media (S) x Type of PGRs (Z)			
S_1Z_0	4.11±1.64abcd	5.33±1.20a	9.67±1.15bcde
S_1Z_1	5.17±2.25abcd	6.44±1.50a	9.00±2.65cde
S_1Z_2	5.44±1.50abcd	6.83±1.26a	11.00±3.00abcde
S_1Z_3	6.61±0.35abc	8.33±1.89a	13.33±0.58ab
S_1Z_4	3.89±1.54acd	5.50±1.32a	9.67±3.21bcde
S_2Z_0	3.17±1.04ad	4.83±2.52a	7.33±0.58e
S_2Z_1	6.94±1.73ab	8.67±1.26a	13.67±0.58a
S_2Z_2	7.06±1.55a	7.22±1.44a	14.00±4.04a
S_2Z_3	5.33±1.76abcd	7.78±2.41a	13.00±3.00ab
S_2Z_4	4.61±1.78abcd	6.72±2.66a	10.33±3.21abcde
S_3Z_0	4.00±2.00bcd	4.44±1.39a	8.33±2.52de
S_3Z_1	4.00±1.73bcd	5.50±2.50a	11.00±4.04abcde
S_3Z_2	5.67±0.67abcd	7.61±1.27a	11.33±2.31abcd
S_3Z_3	5.17±0.29abcd	6.00±0.87a	11.33±2.08abcd
S_3Z_4	7.06±0.42a	8.22±0.84a	12.33±1.15abc

Data are expressed as the mean of determination ± SD in 3 replicates. Within each column, means followed by the same letter are not significantly different among treatments at $p<0.05$.

The type of PGR treatment significantly affected the number of aerial roots (Table 7 and Figure 2B). At 30 and 60 DAP, the highest mean numbers of aerial roots were observed in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, with 6.06 and 5.70 roots, respectively, compared with 4.76 roots in the control (Z_0), representing increases of 27.31% and 19.75%. At 90 DAP, the highest mean numbers of aerial roots were recorded in the 100 ppm IAA (Z_1), 100 ppm BAP (Z_2), and 10% shallot extract (Z_3) treatments, with 11.22, 12.11, and 12.56 roots, respectively, which were 32.94%, 43.48%, and 48.82% higher than the control (Z_0; 8.44 roots). The modified acidic soil medium had no effect on the number of aerial roots in this study.

The interaction between modified acidic soil media and PGR type significantly affected the number of aerial roots at 30 DAP and 90 DAP. At 30 DAP, the highest number of aerial roots (7.06 roots) was observed in both the acid soil + 36 g dolomite + 100 ppm BAP (S_2Z_2) treatment and the acid soil + 250 g goat manure fertilizer + 36 g dolomite + 10% moringa leaf extract (S_3Z_4) treatment. Each represented a 71.78% increase over the acidic soil treatment without PGRs (S_1Z_0), which had 4.11 roots. At 90 DAP, the highest numbers of aerial roots were recorded in the acid soil + 36 g dolomite + 100 ppm IAA (S_2Z_1) and acid soil + 36 g dolomite + 100 ppm BAP (S_2Z_2) treatments, with 13.67 and 14.00 roots, respectively. These values were 41.36% and 44.78% higher than those of the control (S_1Z_0), which had 9.67 roots (Table 8).

Vanilla plant growth is inhibited in soils with low pH. Soils with a pH of 4.0–5.0 are classified as very strongly acidic. The primary factor limiting plant growth in acidic soils is aluminum toxicity. According to Blum et al. (1989), at pH values below 5.0, aluminum dissolves as Al^{3+}, which is toxic to plant roots, rapidly inhibiting root elongation and damaging the root system. Because vanilla roots are shallow and highly susceptible to injury, such conditions can disrupt water and nutrient uptake. Research by Wan et al. (2018) found that Al increases proton (H^+) release and plasma membrane H^+-ATPase activity in roots of *Camellia sinensis*, which is associated with higher Al accumulation and excess cation uptake relative to anion uptake as Al concentration increases. Excessive H^+ release acidifies the rhizosphere and lowers soil pH. In addition, an imbalance between cation and anion uptake disrupts ion homeostasis in root cells. Because elevated H^+-ATPase activity requires substantial energy, and Al accumulation inhibits root cell division and elongation, the combined effects of acidification, nutrient imbalance, and root damage can cause systemic disruption and ultimately reduce plant growth.

The soil's capacity to resist changes in pH (soil buffering capacity) is important for maintaining nutrient availability. The addition of dolomite and organic fertilizers, such as goat manure, as soil amendments improves both the physical and chemical properties of the soil (Głąb et al., 2023; Juniardi et al., 2022). Organic fertilizers such as goat manure increase soil nutrient content, particularly nitrogen, which plays a role in vegetative growth, and improve soil aggregation and microbial activity (Salim et al., 2023). Soarizafy et al. (2024) reported that the application of organic compost derived from agricultural waste (straw and manure) to vanilla at a dose of half a 5-L bucket promoted rapid vine growth, reaching up to 100 cm, which was significantly greater than the control without compost, which reached only 60 cm. In the present study, modified acidic soil media affected vanilla growth, as indicated by total leaf area at 90 DAP. The acid soil + 36 g dolomite (S2) and acid soil + 250 g goat manure fertilizer + 36 g dolomite (S3) treatments resulted in total leaf areas of 377.11 and 403.20 cm², respectively, compared with 270.73 cm² in the control (Z0) (Table 4).

The acidic soil used in this study (pH 4.947) imposed abiotic stress on vanilla plants. PGRs were applied to enhance plant survival under unfavourable environmental conditions, including acidic soil, through various physiological and biochemical mechanisms. Regarding tendrils length, PGR type had a significant effect at 60 DAP, with the 100 ppm BAP treatment (Z_2) producing the highest value (74.50 cm). At 90 DAP, the highest tendrils lengths were recorded in the 100 ppm BAP (Z_2) and 10% shallot extract (Z_3) treatments, at 87.67 and 86.67 cm, respectively. BAP is also known to stimulate lateral shoot growth and branching, thereby directly increasing the number of nodes (Mawaddah et al., 2021).

To properly interpret the growth responses of vanilla cuttings, it is essential first to establish the fundamental soil constraints, as an acidic environment with a pH below 5.5 typically induces high levels of phytotoxic Al^{3+} and Fe^{2+} alongside limited phosphorus availability, which collectively impair root development (Sun et al., 2025). The applied dolomite functions through acid-base neutralization to elevate soil pH and reduce heavy metal ion activity, while the addition of organic matter helps detoxify Al^{3+} primarily through complexation mechanisms where functional groups such as carboxyl and hydroxyl bind with the toxic ions (Lijuan et al., 2025). The integrated application of dolomite and organic amendments has been demonstrated to outperform single applications in mitigating subsoil acidification, a conclusion supported by the synergistic effects observed when dolomite is combined with organic materials based on their titratable alkalinity (Iticha et al., 2024). Even when these chemical improvements did not translate into a statistically significant increase in vanilla stem length during this study, they represent a net improvement in soil quality for sustainable root development and long-term cultivation.

The type of PGR treatment significantly affected the number of internodes, number of leaves, and number of aerial roots at 30, 60, and 90 DAP, whereas total leaf area was affected at 60 and 90 DAP. The most effective PGR treatments were 100 ppm BAP (Z_2) and 10% shallot extract (Z_3). As a cytokinin, BAP promotes cell division and bud formation. As shown in Table 2, the highest number of internodes at 30 DAP was recorded in the 100 ppm BAP treatment (S_2), with 8.85 internodes, whereas the highest value at 90 DAP was observed in the 10% shallot extract treatment (S_3), with 8.56 internodes. At 60 DAP, the highest number of internodes was recorded in the 100 ppm BAP treatment (S_2), with 10.70 internodes. Mahardhini et al. (2023) found that vanilla shoot length did not differ significantly among the 0.6, 1.2, and 1.8 mg L^{-1} BAP treatments, although all three differed significantly from the 2.4 mg L^{-1} treatment. The BAP and control (0 mg L^{-1}) treatments were used for comparison.

The highest number of leaves at 30 DAP was observed in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 8.52 and 8.80 leaves, respectively. At 60 DAP, the highest leaf number was recorded in the 100 ppm BAP treatment (S_2), with 10.26 leaves, whereas at 90 DAP, the highest value was observed in the 10% shallot extract treatment (S_3), with 15.56 leaves (Table 4). Total leaf area was highest at 60 DAP in the 100 ppm BAP (Z_2), 10% shallot extract (Z_3), and 10% moringa leaf extract (Z_4) treatments, with values of 341.21, 328.78, and 363.33 cm², respectively. At 90 DAP, the highest total leaf area was 421.95 cm² in the 10% shallot extract treatment (Z_3). Greater leaf number and leaf area indicate improved plant health and photosynthetic capacity. (Kusbianto et al., 2024). Cytokinin is involved in cell differentiation and organ formation, including in leaves (Kusbianto et al., 2022). The application of synthetic or natural cytokinins mobilizes nutrients to the treatment site. Cytokinin treatment retards chlorophyll degradation and downregulates the expression of genes associated with chlorophyll degradation, thereby delaying leaf senescence (Li Liu et al., 2016).

In addition, the highest numbers of aerial roots at 30 and 60 DAP were recorded in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 6.00 and 5.70 roots, respectively. At 90 DAP, the highest numbers of aerial roots were also observed in the 100 ppm BAP (S_2) and 10% shallot extract (S_3) treatments, with 12.11 and 12.56 roots, respectively. However, the 100 ppm IAA (S_1) treatment also produced a high number of aerial roots, with 11.22 roots (Table 5). The number of aerial roots was also significantly affected by the interaction between the modified acidic soil medium and PGR type at 30 and 90 DAP. Auxin plays a key role in root growth initiation (Aditania et al., 2023). The interaction at 30 and 90 DAP indicates that the stimulatory effect of PGRs on root formation was more pronounced in media with improved physical and chemical properties, allowing the PGRs to act more effectively and efficiently (Juniardi et al., 2022; Salim et al., 2023). Auxins are essential for regulating plant growth and development by governing the formation of primary, secondary, and adventitious roots. The application of rooting hormones enhances adventitious root initiation, improves root quantity and quality, and increases overall rooting percentage (Saini & Anmol, 2024).

(A) (B)

Figure 2. Growth of shoots that will become new internodes (A) and aerial roots (B) of vanilla plants in the first week

(A) (B)

Figure 3. Vanilla plant shoot growth has formed new internodes in the second week (A) and third week (B) of the plant

Acidic soil conditions, characterized by low pH, aluminum toxicity, and nutrient deficiency, represent major constraints to plant growth. PGRs may mitigate these adverse effects through several mechanisms. IAA and BAP enhance stress tolerance by activating antioxidant enzymes that neutralize reactive oxygen species (ROS). Increased activity of these enzymes is important, as superoxide dismutase (SOD) converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), whereas catalase (CAT) further decomposes H_2O_2 into water and oxygen. Aluminum stress in acidic soils increases ROS production, which can damage cell membranes, as reflected by elevated malondialdehyde (MDA) levels. Conversely, a decrease in MDA indicates reduced lipid peroxidation and membrane damage.

Auxins such as IAA plays an important role in plant growth, including root formation and biomass accumulation (Kecis et al., 2023; Ullah et al., 2023). Exogenous application of IAA can increase tolerance to heavy metal stress, such as reducing arsenate toxicity in eggplant roots through endogenous H_2O_2 signaling (Alamri et al., 2021). Auxin carriers such as LAX3 are involved in stress tolerance by affecting stomatal opening and salt tolerance in tobacco(Li, Hong, et al., 2022). Biofertilizer cyanobacteria is also produced IAA and can improve atrazine stress in rice field cyanobacteria (Ahmad et al., 2022). In corn, plant smoke containing IAA and IBA improves root architecture and reduces the effects of the auxin inhibitor 2,3,5-Triiodobenzoic acid (TIBA) (Ullah et al., 2023). Other auxins, such as NAA, help grape growth under drought conditions through physiological and hormonal modifications (Khandani et al., 2024). The protective effects of auxins are generally associated with the modulation of antioxidant enzymes and the suppression of ROS, thereby reducing oxidative damage (Alamri et al., 2021; Khandani et al., 2024).

The application of IAA under Al stress conditions requires caution due to the potential for counterproductive effects. In this study, the IAA treatment did not produce the highest values for all growth variables, namely tendrils length, number of internodes, total leaf area, number of leaves, and number of aerial roots at 30 and 60 DAP; however, it did produce the highest value for the number of aerial roots at 90 DAP. Research on *Citrus limonia* proves that high concentrations of endogenous IAA due to Al stress are correlated with plant susceptibility, where IAA transport-related genes are not differentiated so that IAA accumulates but is not well distributed to root cells (Silva et al., 2019). A study on Arabidopsis thaliana revealed the molecular mechanism, whereby Al inhibits the endocytosis of the PIN2 protein (auxin transporter) by limiting plasma membrane fluidity, which disrupts the basipetal transport of IAA from the transition zone to the root elongation zone, thereby inhibiting cell elongation (X. Li et al., 2018). Based on this evidence, before applying IAA to vanilla plants in acid soil, it is necessary to determine their tolerance level to Al; if vanilla is classified as sensitive, exogenous IAA application carries a high risk of exacerbating Al accumulation in the roots and oxidative damage.

Synthetic cytokinin such as BAP, plays an important role in cell division, differentiation, and shoot regeneration, including in *Vanilla planifolia* (Duri, 2022; L. Li, Li, et al., 2022; Salfiani et al., 2021). Cytokinins such as BAP and cis-zeatin are key regulators that control plant growth and defense systems by regulating antioxidant mechanisms. BAP has been shown to increase salt stress tolerance in grapes by modulating antioxidant genes such as Catalase (CAT) and Ascorbate Peroxidase (APX) (Ahmad et al., 2022; Mahardhini et al., 2023; Ullah et al., 2023). The application of IAA and BAP on Mentha rotundifolia also increases biomass and phytochemical parameters correlated with stress resistance (Alamri et al., 2021; Rivas et al., 2021).

The interaction between IAA and BAP is crucial in stress tolerance, involving the activation of antioxidant defense. Plant hormones form a frontline network that integrates abiotic and biotic stress responses through convergent crosstalk pathways (Ku et al., 2018), which ultimately relies on the management of oxidative stress by the antioxidant system (Ahmad et al., 2022; Khandani et al., 2024; Mahardhini et al., 2023; Ullah et al., 2023). Although direct molecular studies on *Vanilla planifolia* are still limited, this plant is susceptible to stress that induces oxidative stress and naturally possesses an antioxidant system (Alamri et al., 2021; Khandani et al., 2024). Therefore, the exogenous application of IAA and BAP has the potential to enhance the inherent antioxidant system of vanilla to strengthen its resistance to environmental stresses, similar to the mechanisms that occur in other plants.

Natural PGRs derived from natural extracts such as shallot and moringa leaf have the potential to provide synergy because they contain a combination of auxins, cytokinins, and gibberellins simultaneously. These phytohormones also control plant responses to various stimuli such as water availability, light, nutrients, abiotic, and biotic stress. Phytohormones, as plant secondary metabolites, act as chemical messengers that regulate growth, development, reproduction, survival, and senescence; they coordinate signalling pathways for abiotic stress adaptation, and their endogenous fluctuations modulable by exogenous application before or during stress affect all aspects of plant growth (Amoanimaa-Dede et al., 2022). Research by Tini et al. (2022) found that shallot bulbs contain 251.76 ppm IAA; 75.54 ppm kinetin; 23.77 ppm zeatin; and 594.12 ppm GA_3, with the highest GA_3 content compared to banana stems, moringa leaves, sweet corn seeds, and mung bean sprouts. The application of shallot extract to vanilla cuttings resulted in the highest average shoot length (46.60 cm), number of leaves (7.67), and leaf length (8.25 cm) (Rampe et al., 2024). Moringa leaves contain 662.17 ppm IAA; 161.37 ppm kinetin; 55.5 ppm zeatin; and 417.88 ppm GA_3, with the highest levels of IAA and kinetin compared to other sources (Tini et al., 2022). The application of plant growth regulators (PGRs) supports plant growth under specific environmental conditions. PGRs are natural organic compounds in higher plants that are active in small amounts, inducing growth, physiology, and yield in plants (Desta & Amare, 2021).

The discussion comparing synthetic plant growth regulators (BAP and IAA) with natural plant growth regulators, such as shallot extract and moringa leaf extract, is quite interesting, as recent findings provide a clearer picture of the underlying mechanisms. While synthetic IAA is a standard auxin, natural extracts often work through complementary pathways. For instance, shallots are rich in quercetin, a flavonoid that can modulate the distribution of natural auxins in plant tissues (Singh et al., 2021). In comparison, plant-based alternatives such as aloe vera gel can substitute for synthetic auxins in promoting in vitro rooting, indicating that the actions of these complex mixtures often extend beyond a single hormone (Madhushani & Rohanadheera, 2025). Similarly, compared to synthetic cytokinin, Moringa leaf extract owes its efficacy to its zeatin content, a naturally occurring cytokinin promoting cell division and growth. Thus, these extracts mimic or alter natural hormone actions, and their bioactive compounds can also enhance stress resilience, leading to a stronger growth response (Singh et al., 2024; Zia et al., 2021). The comparable or superior performance of these natural extracts on certain parameters likely stems from their multifaceted composition, which provides more balanced hormonal and protective effects.

Based on this mechanism, the application of PGRs to vanilla plants cultivated in acid soil (pH 4.0–5.0) can be specifically targeted. Root system expansion is promoted by IAA for optimal nutrient absorption, while BAP maintains leaf greenness and the photosynthesis rate of tendrils, which influence growth parameters such as tendrils length and internodes of vanilla plants. In addition, natural PGs are an economical alternative with safer, gradual effects. However, specific research on vanilla is still needed to determine the appropriate type of PGR, concentration, and application frequency, considering that plant response to hormones is greatly influenced by species, stress levels, and endogenous hormonal balance (Ahammed & Yu, 2016).

Conclusion

Based on the evaluation of various combinations of modified acidic soil media and types of plant growth regulators (PGRs), the treatment consisting of acid soil + 250 g goat manure fertilizer + 36 g dolomite (S_3) combined with 100 ppm BAP treatment (Z_2) and 10% shallot extract (Z_3) treatments, with consistently produced the highest total leaf area, tendrils length, number of internodes, number of leaves, and number of aerial roots in vanilla cuttings. This specific treatment outperformed all other combinations across the measured growth parameters. Therefore, this treatment can be recommended as an effective strategy for enhancing the establishment of vanilla cuttings in acidic soil conditions. The findings serve as a practical reference for researchers and growers seeking to optimize vanilla cultivation on marginal acidic lands.

Acknowledgments

The authors gratefully acknowledge Universitas Jenderal Soedirman for the financial support and research facilities provided. We wish to express our sincere gratitude to the professors and associate professors of the Agrotechnology Department, Agriculture Faculty for their continuous guidance, critical insights, and support throughout this research project. Their mentorship was invaluable to the successful completion of this study.

References

Aditania R, Sukmawan Y, Same M, Gusta A R. 2023. Pengaruh konsentrasi auksin pada pertumbuhan bibit vanili (*Vanilla planifolia* A.). *Savana Cendana*, 8: 37-42.

Ahammed G J, Yu J Q. 2016. Plant hormones under challenging environmental factors. *Plant Hormones under Challenging Environmental Factors*, 1-269.

Ahmad N, Yasin D, Bano F, Fatma T. 2022. Ameliorative effects of endogenous and exogenous indole-3-acetic acid on atrazine stressed paddy field cyanobacterial biofertilizer *Cylindrospermum stagnale*. *Scientific Reports*, 12: 11175.

Alamri S, Siddiqui M H, Kushwaha B K, Singh V P, Ali H M. 2021. Mitigation of arsenate toxicity by indole-3-acetic acid in brinjal roots: Plausible association with endogenous hydrogen peroxide. *Journal of Hazardous Materials*, 405: 124336.

Amoanimaa-Dede H, Su C, Yeboah A, Zhou H, Zheng D, Zhu H. 2022. Growth regulators promote soybean productivity: A review. *PeerJ*, 10: 1-53.

Ayyubi N N A Al, Kusmanadhi B, Siswoyo T A, Siswoyo T A. 2019. The effect of shallot extract and concentration of coconut water on the growth of shoot-cutting of green deli rose apples (*Syzygium samarangense*). *Berkala Ilmiah Pertanian*, 2: 19-26.

Berndt W L. 2024. Broadening perspective of soil acidity in plant-soil systems. *Journal of Earth & Environment Science*, 1-16.

Blum U, Gerig T M, Weed S B. 1989. Effects of mixtures of phenolic acids on leaf area expansion of cucumber seedlings grown in different pH portsmouth A1 soil materials. *Journal of Chemical Ecology*, 15: 2413-2423.

Brady N C, Weil R R. 2017. *The Nature and Properties of Soils* (15th ed.). Pearson. Boston.

Campbell G S, Norman J M. 1998. *An Introduction to Environmental Biophysics*. Springer. New York.

Desta B, Amare G. 2021. Paclobutrazol as a plant growth regulator. *Chemical and Biological Technologies in Agriculture*, 8: 1-15.

Divakaran M, Suseela Bhai R, Menchaca Garcia R, Aarthi S, Devasahayam S, Nirmal Babu K, Sudarshan M R. 2024. Vanilla. In *Handbook of Spices in India: 75 Years of Research and Development*, 2591-2685. Springer Nature. Singapore.

Duri R D. 2022. Pengaruh kombinasi ZPT IAA dan BAP terhadap pertumbuhan eksplan vanili (*Vanilla planifolia* Andrews) secara in vitro. *Agropross: National Conference Proceedings of Agriculture*, 207-214.

Food and Agriculture Organization of the United Nations (FAO). 2020. *Global Forest Resources Assessment*. FAO. Rome.

Gao Y, Wang M, Shi Y, Yang L, Hu J, Fan K, Shi Y. 2022. IAA accumulation promotes the root growth of tea plants under aluminum. *Agronomy*, 12: 1110.

Głąb T, Gondek K, Marcińska-Mazur L, Jarosz R, Mierzwa–Hersztek M. 2023. Effect of organic/inorganic composites as soil amendments on the biomass productivity and root architecture of spring wheat and rapeseed. *Journal of Environmental Management*, 344: 118628.

Havkin-Frenkel D, Belanger F C. 2018. *Handbook of Vanilla Science and Technology*. Wiley. Hoboken, NJ, USA.

Hidayat A, Mulyani A. 2002. Lahan kering untuk pertanian. Dalam Abdurachman A, Dariah A, Mulyani A (Eds.), *Buku Pengelolaan Lahan Kering Menuju Pertanian Produktif dan Ramah Lingkungan*, 1-34. Pusat Penelitian dan Pengembangan Tanah dan Agroklimat. Bogor.

Iticha B, Mosley L M, Marschner P. 2024. Combining lime and organic amendments based on titratable alkalinity for efficient amelioration of acidic soils. SOIL, 10(1): 33-47.

Juniardi R, Desi Y, Yonny D, Taher A. 2022. Respon pertumbuhan bibit vanili (*Vanilla planifolia*) akibat pemberian bokashi kotoran ayam. *Jurnal Research Ilmu Pertanian*, 2: 1-8.

Kecis H, Bagues M, Abdelouhab Y, Mekircha F, Gali L, Kadi K, Addad D, Nagaz K, Brahmi F, Kouba Y. 2023. Different indole-3-acetic acid and 6 benzyl amino purine concentrations affect biomass, phenolic profile, and bioactivity in *Mentha rotundifolia* L. *Journal of Food Measurement and Characterization*, 17: 4551-4564.

Khandani Y, Sarikhani H, Gholami M, Rad A C, Yousefi S, Sodini M, Sivilotti P. 2024. Exogenous auxin improves the growth of grapevine (*Vitis vinifera* L.) under drought stress by mediating physiological, biochemical and hormonal modifications. *Journal of Soil Science and Plant Nutrition*, 24: 3422-3440.

Kochian L V, Piñeros M A, Liu J, Magalhaes J V. 2015. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annual Review of Plant Biology*, 66: 571-598.

Ku Y S, Sintaha M, Cheung M Y, Lam H M. 2018. Plant hormone signaling crosstalks between biotic and abiotic stress responses. *International Journal of Molecular Sciences*, 19: 3206.

Kusbianto D E, Kurniawan N C, Arum A P, Restanto D P. 2022. Respon induksi tunas tanaman vanili (*Vanilla planifolia* Andrew) terhadap perlakuan konsentrasi BAP dan konsentrasi 2,4-D dengan perbanyakan secara in vitro. *Jurnal Ilmu-Ilmu Pertanian Indonesia*, 24: 82-87.

Kusbianto D E, Ramadhani F, Suud H M, Setiyono S, Meliala S B P. 2024. The effect of planting media composition and concentration of bean sprouts extract on early growth vanilla cuttings (*Vanilla planifolia* Andrews). *Ziraa'Ah Majalah Ilmiah Pertanian*, 49: 322.

Li L, Hong D, An C, Chen Y, Zhao P, Li X, Xiong F, Ren M, Xu R. 2022a. Overexpression of *TaLAX3-1B* alters the stomatal aperture and improves the salt stress resistance of tobacco. *Molecular Biology Reports*, 49: 7455-7464.

Li L, Li X, Yang C, Cheng Y, Cai Z, Nian H, Ma Q. 2022b. GsERF1 enhances *Arabidopsis thaliana* aluminum tolerance through an ethylene-mediated pathway. *BMC Plant Biology*, 22: 1-13.

Li Liu, Li H, Zeng H, Cai Q, Zhou X, Yin C. 2016. Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. *Journal of Plant Growth Regulation*, 35: 366-376.

Liu L J, Liu J H, Zhang H C, Sun C L. 2025. Research progress on alleviation of soil acidification and aluminum phytotoxicity by biochar. Soils, 57(4): 736-743.

Li X, Li Y, Mai J, Tao L, Qu M, Liu J, Shen R, Xu G, Feng Y, Xiao H, Wu L, Shi L, Guo S, Liang J, Zhu Y, He Y, Baluška F, Shabala S, Yu M. 2018. Boron alleviates aluminum toxicity by promoting root alkalization in transition zone via polar auxin transport. *Plant Physiology*, 177: 1254-1266.

Madhushani D, Rohanadheera H. 2025. Evaluation of aloe vera gel as a commercial auxin replacement for in-vitro rooting of sour banana (*Musa acuminata*). *International Symposium on Agriculture and Environment*, 54: 1-2.

Mahardhini A D, Anwar S, Karno. 2023. BAP and kinetin application for in vitro bud induction of vanilla (*Vanilla planifolia* Andrews.) from stem node explants in the initiation stage. *IOP Conference Series: Earth and Environmental Science*, 1246: 012030.

Mawaddah Y, Erawati D N, Donianto M, Ryana W M, Ikanafi'ah A. 2021. The role of cytokinins on shoot multiplication of vanilla explants (*Vanilla planifolia* Andrews.). *Agriprima: Journal of Applied Agricultural Sciences*, 5: 169-179.

Munarso S J, Rahardjo Y P, Sjafrina N, Arianto A, Hadipoentyanti E, Astuti P, Setiadi A, Koeslulat E E, Lintang M M J, Sulistyorini S, Egayanti Y, Elmatsani H M, Djafar M J, Susetyo E B, Lanjar L, Hadipernata M. 2024. From bean to market: exploring the chemical and production dynamics of high-quality Indonesian vanilla. *Frontiers in Sustainable Food Systems*, 8: 1-10.

Murthy G, Umesha K, Smitha G R, Krishnamanohar R. 2010. Effect of growth regulators and bio-inoculants on rooting and growth of vanilla stem cuttings. *Indian Journal of Horticulture*, 67: 90-93.

Pertanian Sekretariat Jenderal Kementrian. 2024. *Outlook Komoditas Perkebunan Vanili*. Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal Kementerian Pertanian. Jakarta.

Rampe H L, Siahaan R, Rampe M J, Moniaga W M, Sakul E H, Tiwow V A. 2024. Morphoanatomy characters, protein and chlorophyll contents of *Vanilla planifolia* Jacks. ex Andrews cuttings to application of natural growth regulators and vegetable elicitors. *Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences*, 67B: 273-282.

Rivas L L P, Rodríguez H L L, Litardo R C M, Almeida I P. 2021. Effect of biostimulants on the growth of vanilla tahitensis in Daule, Ecuador. *Revista Científica Ecociencia*, 8: 25-38.

Saini N, Anmol. 2024. The potential of plant growth regulator (auxin) in enhancing fruit crop propagation via stem cuttings. *International Journal of Advanced Biochemistry Research*, 8: 1001-1007.

Salfiani A, Asri P, Paserang P. 2022. Pengaruh kombinasi IAA (indole-3-acetic acid) dan BAP (6-benzylaminopurine) terhadap inisiasi tanaman vanili (*Vanilla planifolia* Andrews). *Biocelebes*, 16: 157-166.

Salim A, Setyoko U, Madjid A, Asyari H. 2023. Optimization of manage fertilizer media and liquid organic fertilizer of lamtoro leaves on vanilla seed plant. *Jurnal Penelitian Pertanian Terapan*, 23: 47-54.

Singh P, Arif Y, Bajguz A, Hayat S. 2021. The role of quercetin in plants. *Plant Physiology and Biochemistry*, 166: 10-19.

Singh P, Bakshi M, Anmol A. 2024. Natural plant extracts as a sustainable alternative to synthetic plant growth regulators: A review.

International Journal of Advanced Biochemistry Research, 8: 281-287.

Silva C M S, Cavalheiro M F, Bressan A C G, Carvalho B M O, Banhos O F A A, Purgatto E, Harakava R, Tanaka F A O, Habermann G. 2019. Aluminum-induced high IAA concentration may explain the Al susceptibility in *Citrus limonia*. *Plant Growth Regulation*, 87: 123.

Soarizafy F, Rahajason F A, Rasolonjatovo M Z, Randimbiarivelo R H, Rasoanandrasana E. 2024. Crop improvement of the vanilla tree, *Vanilla planifolia*, using organic fertilizer based on rice straw and manure in Mandritsara-Madagascar. *Revue Internationale de la Recherche Scientifique (Revue-IRS)*, 2: 1694-1699.

Sun L, Chen H, Wang Q, Wang W. 2025. Research progress on dolomite improving acidified farmland soil. Multipurpose Utilization of Mineral Resources, 46(2): 121-130.

Suyanto A. 2023. Efektivitas penggunaan pembenah tanah organik dalam meningkatkan pertumbuhan dan hasil tanaman padi (*Oryza sativa* L.) pada tanah sulfat masam. *Jurnal Pangan*, 32: 95-102.

Taiz L, Zeiger E, Møller I M, Murphy A. 2015. *Plant Physiology and Development*. Sinauer Associates. Sunderland.

Tao L, Xiao X, Huang Q, Zhu H, Feng Y, Li Y, Li X, Guo Z, Liu J, Wu F, Pirayesh N, Mahmud S, Shen R F, Shabala S, Baluška F, Shi L, Yu M. 2023. Boron supply restores aluminum-blocked auxin transport by the modulation of PIN2 trafficking in the root apical transition zone. *Plant Journal*, 114: 176-192.

Tini E W, Sakhidin, Saparso, Haryanto T A D. 2022. Kandungan hormon endogen pada tanaman hortikultura. *Jurnal Galung Tropika*, 11: 132-142.

Ullah G, Ibrahim M, Nawaz G, Khatoon A, Jamil M, Rehman S U, Ali E A, Tariq A. 2023. Plant-derived smoke mitigates the inhibitory effects of the auxin inhibitor 2,3,5-triiodo benzoic acid (TIBA) by enhancing root architecture and biochemical parameters in maize. *Plants*, 12: 2604.

Unit D, Garden R B. 2023. Exploring the potential use of *Moringa olifera* Lam and *Azadirachta indica* additives in Orchid tissue culture.

Wan Q, Mi Y, Yang Y, Renkou X, Li X. 2018. Aluminum-enhanced proton release associated with plasma membrane H+-adenosine triphosphatase activity and excess cation uptake in tea (*Camellia sinensis*) plant roots. *Pedosphere*, 28: 804-813.

Wang S, Ren X, Huang B, Wang G, Zhou P, An Y. 2016. Aluminium-induced reduction of plant growth in alfalfa (*Medicago sativa*) is mediated by interrupting auxin transport and accumulation in roots. *Scientific Reports*, 6: 1-13.

World Population Review. 2024. Vanilla production by country. *World Population Review*. Diakses dari https://worldpopulationreview.com/country-rankings/vanilla-production-by-country.

Wu H, Hu J, Shaaban M, Xu P, Zhao J, Hu R. 2021. The effect of dolomite amendment on soil organic carbon mineralization is determined by the dolomite size. *Ecological Processes*, 10: 8.

Zakiah Z, Turnip M. 2023. Improving the growth and adaptation of the black orchid plantlet (*Coelogyne pandurata* Lindl) in various growing media by giving plant extracts as biostimulants at the acclimatization stage. *J Juatika*, 5: 301-310.

Zia U A, Furqan M, Rashid E, Gul S, Dhaku H N, Murtaza G, Haider L, Haider S Z, Iqbal M, Aslam S. 2021. Allelopathic effect of moringa oleifera leaf extract on growth of maize (*Zea mays*). *Plant Cell Biotechnology and Molecular Biology*, 22: 12-23.