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Planta Tropika

Planta Tropika focuses related to various themes, topics and aspects including (but not limited) to the following topics Agro-Biotechnology, Plant Breeding, Agriculture Waste Management, Plant Protection, Soil Science, Post Harvest Science and Technology, Horticulture. Planta Tropika published two times a year (February and August) by Universitas Muhammadiyah Yogyakarta in collaboration with Indonesian Association of Agrotechnology / Agroecotechnology (PAGI) and Perhimpunan Agronomi Indonesia (PERAGI). The subscriptions for one year : IDR 350.000.

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Editorial

Journal of Planta Tropika ISSN 0216-499X published by Study Program of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, is journal presenting scientific articles of agricultural science (Journal of Agro Science). With full sense of gratitude to the Almighty Allah, Volume 12 Number 2 for the year of 2024 has been published.

In this edition, Journal of Planta Tropika presents eight research articles in the field of Agro sciences comprising soil and plant nutrition, plant protection, plant breeding, plant physiology. The scientific articles discuss about:

(1) Physiological Response of Two Amaranth Varieties (*Amaranthus tricolor* L.) to Urea Applications, (2) Effects of Water Clover Density and Submerged NPK Fertilizer on Rice Production, (3) Goatweed Flower Extract (*Ageratum conyzoides* L.) as a Botanical Insecticide for Pest Control *Crocidolomia binotalis* Z., (4) Estimating SPAD, Nitrogen Concentration, and Chlorophyll Content in Rice Leaves using Calibrated Smartphone Digital Image, (5) Floral Morphological Variation in Black Pepper (*Piper nigrum* L.) Varieties and Hybrid Lines, (6)Abundance, Attack Intensity, and Distribution of *Spodoptera frugiperda* J.E. Smith in Kulon Progo, Yogyakarta, (7) Physiological Traits of Vanilla Plant (*Vanilla planifolia* Andrew) in Various Types of Shade Trees, (8) Quality Coefficient on Gene Differentiation and Phenotype: Clone Assessment of *Saccharum officinarum* Linn.

The editors would like to thank the authors, reviewers, executive editors, leaders and LRI UMY for their participation and cooperation. Our hope, this journal can be useful for readers or be a reference for other researchers and useful for the advancement of the agriculture.

Editors

GUIDE FOR AUTHORS

TYPE OF PAPERS

PLANTA TROPIKA receives manuscripts in the form of research papers in Bahasa Indonesia or English. The manuscript submitted is a research paper that has never been published in a journal or other publication.

SUBMISSION

The submission of the manuscript is done through our journal website http://journal.umy.ac.id/index.php/pt/index. If you need information regarding the process and procedure for sending the manuscript, you can send it via email at plantatropika@umy.ac.id. Editor's address: Program Studi Agroteknologi, Fakultas Pertanian, Universitas Muhammadiyah Yogyakarta, Jl. Brawijaya, Tamantirto, Kasihan, Bantul, Telp (0274) 387646 psw 224, ISSN: 2528-7079.

ARTICLE STRUCTURE

The submitted manuscripts should consist of 15-20 pages of A4 size paper with 12-point Times New Roman fonts, 1.5 spacing with left-right margin and top-bottom of the paper is 2.5 cm each. All manuscript pages including images, tables and references should be page-numbered. Each table or picture should be numbered and titled.

The systematic of the manuscript writing is as follows:

TITLE : The title should be written in a concise but informative manner describing the content of the research. Avoid abbreviations or formulas. Font style is Tahoma 18pt.

AUTHOR NAMES : The author names should be written in lowercase letters (only the first letter of the words is written in uppercase) and should be written from the first author and followed by the others along with the marker of each author's affiliation.

AUTHOR AFFILIATIONS : The author affiliation should be written in lowercase letters (only the first letter of the words is written in uppercase) and it is written according to the order of the number marker of each author's affiliation.

EMAIL : Please list one of authors' email address used for paper's correspondence.

ABSTRACT : Abstract written in English. Abstract appears after the Title in the manuscript. Abstract must be solid consisting of an introduction and objectives, research methods, results, and conclusions. Abstract must be written in one paragraph. Libraries are not allowed to be written in the abstract section. Writing abbreviations should be avoided. Abstract consists of 150-200 words followed by keywords. Abstract is written in justify.

INTRODUCTION : This section describes: (i) the general background of the research (concise and clear), (ii) a review of the results of previous research that is relevant and up to date, (iii) clearly provides a statement of novelty (gap analysis) which contains the urgency and novelty of the research, as well as the objectives of the study. The introduction is written without numbering and / or pointers. The introduction is written in 750-1000 words.

MATERIALS AND METHOD : This section should state the time and place for conducting the research. All materials and methods used such as chemicals for analysis, treatment and experimental design must be clearly and concisely stated. The use of methods should be based on previous research. Materials and methods should be written in 400 - 600 words. Subsection heading might be applied if there are many sections appeared.

RESULT AND DISCUSSION : In this section the results and discussion must be written in one section. Both must be written in a continuous manner starting from the main results to supporting results and completed with discussion. The unit of measurement used must follow international standards. Equations are written in Times New Roman or Symbol fonts. If there are several equations, give each equation a number in sequence and place it on the far right, namely (1), (2), and so on. The use of signs is allowed to make equations more concise. Italic fonts are used for variables, bold for vectors. Example:

$$\alpha + \beta = \chi \qquad (1)$$

Results and discussion should be written between 2500 - 3000 words. It can be grouped in several sub-section heading.

CONCLUSION : In this section, conclusions must be written clearly and concisely referring to the research results to answer the research objectives or hypotheses, and not repeat the discussion. Conclusions are written critically, logically and honestly based on existing facts, and be careful if there are generalizations. This section is written in paragraph form, not using numbering or bullets. Results and discussion should be written between 40-80 words.

ACKNOWLEDGEMENT : This section is used if needed. This section is provided for authors to express their gratitude to either the party who funded the research, facilities, or suggestions; also for a statement if the article is part of a thesis / dissertation.

REFERENCES : All libraries used should be noted in this section. The use of bibliography writing style follows the style of the American Psychological Association (APA) 6th Edition and is arranged in alphabetical order (https://apastyle.apa.org/style-grammar-guidelines/references/examples). Manuscripts must use a minimum of 30 references and 80% come from journals (**for the new submission starting January 2024**). The majority of the literature used consists of primary literature (published in the last 5 years). Unpublished data and personal communications are not allowed to be cited. Between one library and the next one separated by one space. It is mandatory to include the DOI of cited references, if available. **Author are suggested to use reference tools, such as Mendeley, Zotero and Endnote.**

EXAMPLES ON HOW TO WRITE REFERENCES

References are written in alphabetical order according to the rules below:

REFERENCE TO A BOOK

Pessarakli, M. (2001). *Handbook of Plant and Crop Physiology* (4th Ed.). CRC Press.

REFERENCE TO A JOURNAL PUBLICATION

Aiman, U., Tantriati, T., & Sriwijaya, B. (2017). Pemberian Macam Konsorsium Bakteri Hasil Isolasi Tumbuhan Pantai pada Kangkung (*Ipomoea reptans* Poirs.). *Planta Tropika*, *5*(1), 1–6. https://doi.org/10.18196/pt.2017.065.1-6

REFERENCE TO A THESIS/DISSERTATION

Miranda, C. (2019). *Exploring the lived experiences of foster youth who obtained graduate level degrees: Self-efficacy, resilience, and the impact on identity development* (Publication No. 27542827) [Doctoral dissertation, Pepperdine University]. PQDT Open. https://pqdtopen.proquest.com/doc/2309521814.html?FMT=AI

REFERENCE TO AN ARTICLE IN PROCEEDING

Sarjiyah, Setiawan, D. A., & Rineksane, I. A. (2021). Shallot extract enhance root growth in crystal guava (*Psidium guajava*) stem cuttings. *IOP Conference Series: Earth and Environmental Science*, *752*(1), 012050. https://doi.org/10.1088/1755-1315/752/1/012050

REFERENCE TO A REPORT GOVERNMENT

Ministry of Agriculture. (2019). *Taking time: Support for improve agriculture product* (MOA Publication No. 18-2059). Indonesia. Department of Crop Production, Ministry of Agriculture. https://psp.pertanian.go.id/

TABLE AND FIGURE FORMATTING : Table labels are placed on top of the table, while image labels are at the bottom of the image. When referring to a table, write down a specific table, for example Table 1. Examples of writing tables and figures are as follows.

Table 1. Table format (Verdana 10pt, align left, space 1, spacing before 0 pt, after 0 pt, without a period)

Head of table	Head of table column	
	Sub-column header	Sub-column header
Contents	Contents	Contents

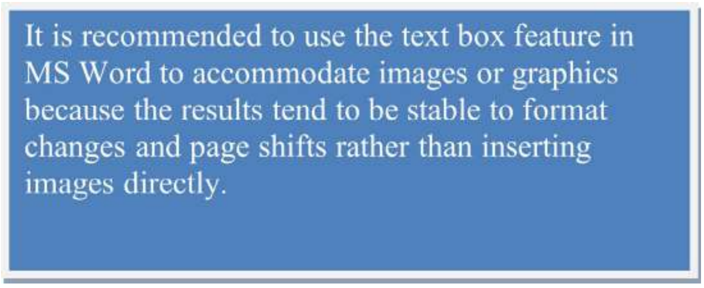

Figure 1. Examples of image captions (Verdana 10pt, center, space 1, spacing before 0 pt, after 0 pt, without a period)

Physiological Response of Two Amaranth Varieties (*Amaranthus tricolor* L.) to Urea Applications

10.18196/pt.v12i2.20933

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ABSTRACT

Amaranth, a highly nutritious leaf vegetable, requires adequate nutrients, especially nitrogen fertilizers, to grow well and produce an optimal yield. Urea is commonly used to promote the growth of amaranth plants. This study examined the physiological responses of two varieties of amaranth to the application of urea fertilizer. This study examined the physiological responses of two varieties of amaranth to the application of urea fertilizer. This investigation was conducted in July - August 2023 in Sukosari Village, Jumantono, Karanganyar. The study utilized a completely randomized design (CRD) with two treatment variables. The first variable consisted of a variety of amaranth, including green (B1) and red (B2) varieties. The second variable in the study was the dosages of urea, including 0, 50, 100, 150, 200, 250, and 300 kg.ha-1. The study observed no correlation between various varieties of amaranth and the dose of urea applied in terms of plant growth and physiological activities. There was no discernible impact on the growth or physiological activity of both varieties of amaranth when urea fertilizer was applied. Both varieties of amaranth showed similar physiological responses to urea fertilization. However, the effect of adding urea fertilizer was to enhance the vegetative growth of amaranth plants.

Keywords: Amaranth; Analysis; Fertilizer; Growth; Nitrogen

INTRODUCTION

Amaranth (*Amaranthus sp.*) originated in Tropical America and then spread to various parts of the country. Amaranth was originally an ornamental plant but is now known as a popular vegetable high in protein and vitamins. Amaranth is a plant rich in protein and a valuable source of vitamins A, B, C, and fiber. Additionally, the leaves of amaranth contain significant amounts of oxalic acid. Furthermore, amaranth also includes minerals, iron, magnesium, phosphorus, calcium, and charcoal hydrate in the form of cellulose (Suarjana et al., 2019). The two most consumed and cultivated amaranth types in Indonesia are green and red, with similar nutritional content. Amaranth contains all the nutrients such as carbohydrates, proteins, fats, vitamins A, B1, B3, B9, C, and minerals such as calcium, sodium, and potassium (Mahmud et al., 2018). The high nutritional content of amaranth makes it one of the sources of leaf vegetables that are beneficial to the body, so it is widely cultivated both commercially and at the household level (Ghufron et al., 2023).

Article History
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The cultivation of amaranth plants is quite simple, with factors that must be considered, such as the availability of nutrients necessary for plant growth. The availability of nutrients is crucial in increasing plant growth and yield. The nutritional needs of plants in cultivation activities are met through fertilization (Nurrudin et al., 2020). During its growth and development, amaranth plants depend highly on nitrogen availability. Amaranth is a leaf vegetable, where the leaves and stems are consumed, so it requires a large amount of nitrogen during its growth and development. Nitrogen is a vital element that serves an indispensable function in plant vegetative stages through its provision of assimilation, which is utilized in the development of organ structures (including roots, stems, and leaves) and metabolic processes (Annisa et al., 2018; Rahmani et al., 2020).

Nitrogen requirements during cultivation are met through fertilization. A nitrogen fertilizer that is widely applied in vegetable cultivation is urea. The high nitrogen content in urea fertilizer supports plant growth and development. Urea fertilization produces greener, fresher, and more lush plant leaves. Nitrogen plays a role in the production of chlorophyll, the green colour found in leaves. The high chlorophyll level will increase the photosynthesis process and encourage the formation of vegetative parts like plant height, count of tillers, count of branches, and others (Kogoya et al., 2018). Fertilization with chemical fertilizers (inorganic) with high nutrient content can meet the nutrient needs of amaranth, such as N from urea fertilizer, which has a nitrogen content of 46%, which is important for vegetative growth (Ramadhanty et al., 2024). Nitrogen is a crucial element for plants, serving as the main nutrient. It is a fundamental component of proteins, which are made up of amino acids. These proteins catalyze chemical reactions, transport electrons, and produce chlorophyll. Chlorophyll is essential for photosynthesis, which occurs in various important parts of the plant's body. Nitrogen imparts plants with their characteristic dark green hue, facilitating the proliferation and maturation of foliage, stems, and other non-reproductive components.

Furthermore, nitrogen also promotes the development of roots. Nitrogen is important in numerous physiological processes that impact a plant's growth and productivity (Zayed et al., 2023). Efforts have been made in previous research to increase the yield of amaranth plants. The height of the plant, leaf area, and count of leaves are the main focus of various nitrogen fertilizer applications during plant growth and development. According to Gorung et al. (2022), a 200 kg.ha^{-1} urea dose can enhance amaranth growth, including plant height, leaf count, and total weight. Budiyani et al. (2022) found that the application of urea fertilization significantly interacted with green amaranth varieties to improve the total fresh weight of plants by up to 75.18%. Nevertheless, further investigation is required to thoroughly examine the physiological reaction of amaranth plants when exposed to nitrogen fertilizer during their growth and development. This study examined the physiological responses of two amaranth varieties to urea fertilizer.

MATERIALS AND METHOD

Study Area

The experiment was conducted at the experimental area of the Faculty of Agriculture, Sebelas Maret University in Sukosari Village, Jumantono District, Karanganyar Regency, from July to August 2023, with an altitude of 177.7 meters above sea level at the coordinates of -7°37'50.496"S and

110°56'55.116"E. Laboratory analysis was performed at the Laboratory of Plant Physiology and Biotechnology, as well as the Laboratory of Ecology and Crop Production Management, Faculty of Agriculture, Sebelas Maret University.

Experimental Design

The experiment was arranged in a two-factor factorial completely randomized design (CRD), consisting of amaranth varieties and urea doses as factors. The amaranth varieties used were certified seeds widely cultivated and composed of two amaranth plants: the Maestro variety of green amaranth and the Mira variety of red amaranth. The doses of urea consisted of 7 levels, namely 0, 50, 100, 150, 200, 250, and 300 $kg.ha^{-1}$ based on nitrogen fertilization recommendations of 100 $kg.ha^{-1}$ to 200 $kg.ha^{-1}$ (Gorung et al., 2022; Hendra et al., 2021). From each treatment, 14 combined treatments were obtained, which were then made into three replications to obtain 42 experimental units.

Research Practices

The research stages included land preparation, seed germination, transplanting, fertilization, watering, pest control, and harvesting. The seeds were germinated in seedling trays for 14 days. Amaranth seedlings were transferred from the seedling tray and planted in the planting media in polybags with one seedling per polybag (Annisa et al., 2018). 25 cm x 30 cm polybags were placed atop gallons for planting. The distance between the gallons was 30 cm x 30 cm. The planting medium was soil mixed with dolomite at 0.09 g/polybags. Amaranth plants require a pH of 6-7 during their growth, so adding dolomite increases the pH of the soil to reach a neutral reaction. The dolomite application increases soil pH because dolomite is used as a substitute for cations such as Al^{3+}, which can acidify the soil. An increase in base cations, neutralising soil pH balances a decrease in the concentration of Al^{3+} dissolved in the soil (Iswara & Nuraini, 2022). KCL and TSP fertilizers were applied after seven days after transplanting (DAT) at a dose of 100 $kg.ha^{-1}$ and 50 $kg.ha^{-1}$, respectively, while urea fertilizer application was given 7 and 14 days after transplanting (DAT) with a dose ratio 50:50 for each treatment dose. Watering was done twice daily while handling plant-disrupting organisms was done manually.

Observation

The research observations included several variables, namely plant growth (plant fresh weight, leaf area index, specific leaf weight, and specific leaf area) and plant physiological activity (photosynthetic rate, transpiration rate, stomatal conductance, and leaf chlorophyll content). Plant fresh weight (g) was obtained by weighing all plant parts from each treatment carried out at harvest time, namely at the age of 28 DAT. Weighing was carried out in the condition of plants cleaned from the soil using analytical scales (accuracy ± 0.01). Leaf area index, specific leaf weight, and specific leaf area were calculated using the equation from (Gardner et al., 1991) as follows:

$$LAI = \frac{LA}{A} \quad (1)$$

LAI = leaf area index, LA = total leaf area, and A = area. Specific leaf weight was calculated with the following equation:

$$SLW = \frac{Lw}{LA} \quad (2)$$

SLW = specific leaf weight, Lw = fresh leaf weight, and LA = leaf area, while the specific leaf area was calculated using the following equation:

$$SLA = \frac{LA}{Lw} \quad (3)$$

SLA = Specific Leaf Area, LA = leaf area, and Lw = leaf weight.

Photosynthetic rate, transpiration rate, and stomatal conductance were measured using a Portable Plant Photosynthetic Meter (Model NY-1020, Nanbei instrument limited) on the third fully opened leaf between 10:00 a.m. and 1:00 p.m. under sunny conditions (not cloudy) (Boy et al., 2022). The chlorophyll content of leaves was determined utilizing the Arnon (1949) method (Rahayu et al., 2021) by pulverizing 0.5 grams of fully opened amaranth leaves in a mortar, followed by the addition of 10 ml of 80% acetone. Subsequently, the solution was passed through a piece of filter paper. The filtrate was introduced into the cuvette to the designated limit to determine the absorbance. A spectrophotometer measured the absorbance at two specific wavelengths (λ) of 645 and 663 nm. The calculation for chlorophyll content was as follows:

$$\text{Chlorophyll a} = (12.7 \times A663 - 2.69 \times A645) \times (10\ \text{ml}/1000 \times 0.5\ \text{g}) \quad (4)$$

$$\text{Chlorophyll b} = (22.9 \times A645 - 4.68 \times A663) \times (10\ \text{ml}/1000 \times 0.5\ \text{g}) \quad (5)$$

$$\text{Total Chlorophyll} = (20.2 \times A645 + 8.02 \times A663) \times (10\ \text{ml}/1000 \times 0.5\ \text{g}) \quad (6)$$

Stomatal openings and stomata density were quantified using the replica method (Wolf et al., 1979). First, the surface of the leaf samples on each side was cleaned using tissue to remove dust, dirt, and debris. The lower leaf surface (abaxial) that had been cleaned was smeared with a cuticle and left for approximately 10–15 minutes until the cuticle dried. The latex spread that had dried was peeled off / taken slowly directly, or the latex spread that had dried was plastered with tape and flattened, then the tape was taken and attached to the glass object, flattened, and labeled according to the treatment. The number of stomata and the width of stomatal openings were observed using a binocular microscope with weak (100x) and strong (400x) magnification and documented using an Optilab microscope camera (Prastika et al., 2023).

Data Analysis

The data were analyzed using analysis of variance and then tested using the Duncan Multiple Range Test (DMRT) at a significance level of $\alpha = 5\%$. The analysis was conducted to determine the presence of a highly significant effect on the variables observed.

RESULTS AND DISCUSSION

The results showed no significant interaction ($p > 0.05$) between urea doses and amaranth varieties for all observed variables. Doses of urea and amaranth varieties showed results that were not significantly different ($p > 0.05$) between treatments. These results indicate that the doses of urea given to the two amaranth varieties have not been able to increase amaranth growth significantly. In general, adding urea as a nitrogen source spurs the vegetative growth of amaranth plants. Nitrogen content in the growing medium is too low (Table 1), so adding urea fertilizer as the sole nitrogen source in amaranth cultivation has not met the nitrogen requirements needed to obtain maximum

Table 1. Soil Chemical Analysis Results

Soil Chemical	Results	Units	Marking *
pH	5.97	-	Slightly acidic
Potential K	7.18	$mgK_2O/100g$	Extremely low
Available P	1.77	ppm	Extremely low
CEC	32.08	cmol(+)/kg	High
Organic C	3.64	%	High
Total N	0.40	%	Extremely low
Texture			
Loam	8.63	%	Loam
Silt	48.36	%	
Sand	43.01	%	

Source: Soil analysis results of BSIP Environmental Agriculture, Pati, and Soil Laboratory, UGM. Marking based on Petunjuk Teknis Analisa Tanah, Tanaman, Air dan Pupuk, Balai Penelitian Tanah (2009).

Table 2. Fresh Weight (g), Leaf Area Index, Specific Leaf Weight and Specific Leaf Area

Treatment	Fresh Weight (g)	Leaf Area Index	Specific Leaf Weight ($g.cm^{-2}$)	Specific Leaf Area ($cm^2.g^{-1}$)
Amaranth				
Green	4.453 a	0.40 a	0.021 a	985.830 a
Red	3.874 a	0.31 a	0.021 a	521.201 a
Urea ($kg.ha^1$)				
0	2.688 a	0.27 a	0.019 a	614.908 a
50	3.653 a	0.25 a	0.021 a	531.856 a
100	4.216 a	0.38 a	0.018 a	516.265 a
150	3.966 a	0.38 a	0.018 a	619.698 a
200	5.234 a	0.37 a	0.019 a	574.551 a
250	3.551 a	0.28 a	0.025 a	554.878 a
300	5.836 a	0.56 a	0.024 a	589.788 a
Interaction	-	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Duncan's Multiple Range Test at 0.05 level of significance.

yields. Prakoso et al. (2018) stated that the same N content in the growing media used affects the total N content so that the N absorbed during the addition of urea fertilizer is thought to be not appropriately accumulated, which results in the utilization of nitrogen by plants not being optimal. In addition, the nitrogen in urea fertilizer is susceptible to rapid loss via evaporation and leaching due to its rapid release, whereas nitrogen is mobile. Without sufficient N, plant growth will be better; however, plant growth and development will only be optimal if plant needs are fulfilled (Faqih et al., 2019; Rahayu et al., 2021). Green amaranth produced a higher fresh weight of stalks; the red amaranth exhibited a lower leaf area index and a specific leaf area than the other amaranth varieties. However, the specific leaf weight of both amaranth varieties showed similar results, with no significant effect observed ($p > 0.05$) (Table 2). It has been observed that plant genetics play significant roles in the superior growth of green amaranth over red amaranth. Plant growth is influenced by a variety of factors, including environmental conditions, genetics, and cultivation techniques. The use of various varieties is the application of genetic factors. Genetics of a variety will significantly affect the potential yield. Crop management factors include determining the amount and timing of fertilizer application. Genetic influences and environmental conditions can cause plants to have different phenotypes. Each different gene in each variety is expressed in different characters. Environmental factors continue to influence the appearance of genes, so similar plants are often obtained but with different characteristics (Apriliani, 2022).

In the treatment of urea doses given, the highest average fresh weight of stems and leaf area index were obtained from the addition of the urea dose of 300 $kg.ha^{-1}$; the greatest specific leaf weight was observed at urea doses of 250 $kg.ha^{-1}$; while the highest specific leaf area was observed at urea doses of 150 $kg.ha^{-1}$. Nevertheless, these values showed no substantial variation across all growth variables. The interaction between urea fertilizer and other soil nutrients affects fertilizer's effectiveness in increasing plant growth. The different doses of urea used without being followed by differences in K and P fertilizers are thought to result in an imbalance of nutrients that amaranth plants need, so they cannot increase growth optimally. KCL and TSP fertilizers were applied at the same doses to all plants based on amaranth fertilizer recommendations with a ratio of N: P: K of 100:100:50 kg/ha, so it is better if the increase in N dose is also followed by an increase in K and P fertilizers so that the nutritional needs of plants can be met. According to Satriawi et al. (2020), N is a nutrient that plays a significant role in photosynthesis. If the photosynthesis process runs well, photosynthesis will increase, increasing plant growth and fresh weight. The K nutrient element is a nutrient that plays a role in opening and closing the stomata, so if the plant lacks the K element, it will not be able to carry out the photosynthesis process optimally and result in a decrease in the fresh weight produced by the plant (Marian & Tuhuteru, 2019). Nutrient P is a nutrient that helps activate enzymes in the photosynthesis process so that the photosynthesis process can run well. The fresh weight obtained was similar, presumably due to the condition of amaranth plants, which mostly have thin and broad leaves, so even though the plants have many leaves and leaf areas, it does not affect the fresh weight of amaranth plants. The thinness of these leaves is also an indicator that the leaves have low water content and low mesophyll tissue. Leaf tissue is mesophyll. Leaf mesophyll tissue is the most active part of photosynthesis, and mesophyll forms plant assimilates. Therefore, the fresh weight produced is also low even though it has many leaves (Prakoso et al., 2018; Wahyuningtyas et al., 2022).

The whole weight of plant components not harvested is known as the fresh weight of stover. Green amaranth had a higher average fresh weight than red amaranth, although the differences were not statistically significant ($p>0.05$). Green amaranth has longer and more roots than red amaranth (Telaumbanua et al., 2023). The increase in fresh weight of stover is correlated with the increase in LAI. The addition of urea doses causes sufficient nitrogen requirements for plant growth, thus increasing the leaf area index of plants (Manurung & Nurchayati, 2020). A measure expressing the relationship between the total area of a plant's leaves and the ground acres it occupies is called the leaf area index, also referred to as LAI. The higher the LAI, the more photosynthesis the plant can carry out, which means that the leaf surface available for photosynthesis is more comprehensive in producing assimilates for plant growth. The increase in leaf area is caused by nitrogen released by urea fertilizer, which increases plants' vegetative growth, such as roots, stems, and leaves (Mondal et al., 2019). If nitrogen nutrient requirements are met, plants perform photosynthesis, producing organic compounds that are converted into energy during respiration. Plants then put this energy to use for their growth, which manifests in an increase in the number of leaves, leaf area, and the overall mass of plants (Apriliani, 2022). The LAI increase indicates plants' response to the addition of urea doses the increase in urea dose results in greater availability of nitrogen required by plants for growth. The leaves' ability to capture sunlight increases with a greater leaf area so that the photosynthesis process and the energy produced are also greater, ultimately increasing the plant's total dry weight (Lutfiah & Pratiwi, 2021).

Specific leaf weight and a specific leaf area are test parameters used to measure the thickness of plant leaves. After analyzing the variance, the specific leaf weight and specific leaf area did not show any significant variations across different amaranth varieties and urea doses. The average specific leaf weight in both varieties gave the same result of 0.021 $g.cm^{-2}$, while the urea doses produced an average specific leaf weight of 0.18 to 0.25 $g.cm^{-2}$ (Table 2).

Plants' growth and metabolism depend entirely on the nitrogen supply. Nitrogen is a crucial mineral that stimulates the development of plant structures such as stems, leaves, and roots. It also plays a vital role in producing various biomolecules, including proteins, amino acids, nucleic acids, coenzymes, and chlorophyll. Chlorophyll is particularly significant due to its crucial function in photosynthesis (Mengesha, 2021). Larger applications of urea can increase the nitrogen content available to plants that can be used in their growth. Plants absorb nitrogen to produce more vegetative parts, particularly leaves. Nitrogen is crucial in the formation of amino acids, the fundamental building blocks of proteins, so the greater the absorption of nitrogen by plants will increase the formation of the number and size of plant cells (Handayani et al., 2020; Kogoya et al., 2018; Yadav et al., 2022; Zaman et al., 2022).

Leaf thickness measurements determine the condition of the leaves produced during plant growth. The specific leaf weight (SLW) and the specific leaf area (SLA) have an inverse relationship. The specific leaf area (SLA) and/or specific leaf weight (SLW) of thick leaves exhibit lower values, while thin leaves have higher and/or lower values, respectively (Dahu, 2022). Low specific leaf weight values indicate that plants are lighter tolerant than plants with high specific leaf weight values. Plants

Table 3. Photosynthetic Rate, Transpiration Rate, Stomatal Conductance, Number of Stomata and Stomatal Opening Width

Treatment	Photosynthetic Rate ($\mu mol.m^{-2}.s^{-1}$)	Transpiration Rate ($\mu mol.m^{-2}.s^{-1}$)	Stomatal Conductance ($\mu mol.m^{-2}.s^{-1}$)	Number of Stomata	Stomatal Opening Width (μm)
Amaranth					
Green	16.894 a	0.249 a	7.762 a	27.714 a	45.343 b
Red	10.929 a	0.238 a	7.482 a	26.048 a	34.352 a
Urea ($kg.ha^{-1}$)					
0	10.973 a	0.203 a	6.256 a	30.167 a	59.867 b
50	10.363 a	0.263 a	8.120 a	33.000 a	40.133 ab
100	6.588 a	0.352 a	10.990 a	31.000 a	40.500 ab
150	19.612 a	0.286 a	8.673 a	22.500 a	31.233 a
200	20.966 a	0.181 a	5.628 a	22.167 a	35.967 a
250	20.117 a	0.205 a	6.896 a	20.167 a	34.133 a
300	8.760 a	0.214 a	6.788 a	29.167 a	37.100 a
Interaction	-	-	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Dun can's Multiple Range Test at 0.05 level of significance.

with a small specific leaf weight usually have wider leaves with thin leaf thickness. Palisade cells that are formed are few and have a short cell size. Greater light capture is possible in wide and thin leaves, resulting in the transmission of captured light to the lower area of the leaf, which can take place quickly so that the photosynthesis process runs more optimally (Saputri et al., 2019; Sumardi et al., 2019). Leaf thickness is related to the rate of photosynthesis because leaf thickness describes the photosynthetic organelle unit. Thick leaves have more chloroplasts per unit leaf area, resulting in a greater ability to intercept light and decompose CO_2 than thin leaves (Sitompul & Guritno, 1995). In this study, leaf thickness measured through a specific leaf area and a specific leaf weight did not show significantly different results ($p > 0.05$).

Plant physiological activity can be known from several test parameters, including photosynthesis rate, transpiration rate, stomatal conductance, the number of stomata, and stomatal opening width. According to the analysis of variance (refer to Table 3), there was no interaction effect ($p > 0.05$) of amaranth varieties and urea doses on all plant physiological activities. Green amaranth's physiological activity is not significantly different ($p > 0.05$) from red amaranth except in the width of stomatal openings. The treatment of urea doses showed no significant effect ($p > 0.05$) on all plant physiological activities.

Leaves are an essential component for plants' growth and productivity because they are where photosynthetic activities take place, namely the physiological activity of food synthesis for plant growth, so the area and number of plant leaves affect photosynthetic activities. The greater number of leaves will increase the photosynthetic reaction as a result of the more food produced. Otherwise, the small number of leaves will cause a low rate of photosynthesis in plants (Wahyudi et al., 2018). The wider the plant leaves, the higher the rate of photosynthesis, which is because the wider the leaf surface can absorb sunlight for photosynthesis (Yuniansyah et al., 2022).

The availability of nitrogen affects the rate of plant photosynthesis. Plant nutrients that are sufficiently available will allow the leaves to meet their needs for photosynthesis. Lack of nitrogen will affect photosynthesis because nitrogen is an important ingredient of chlorophyll, so if its availability is limited, chlorophyll cannot be formed, which results in inhibition of the photosynthesis process (Gardner et al., 1991). Sitompul & Guritno (1995) state that the speed of plastochron formation (the time needed to develop each new leaf) and filochron emergence (the time required for each new leaf to form) affect the formation of plant leaves. Temperature, light intensity, and nitrogen (N) availability are the main factors that influence leaf growth. In this study, the average photosynthetic rate of green amaranth was higher than that of red amaranth, but the chlorophyll content of red amaranth was higher than that of green amaranth. The higher chlorophyll content in red amaranth is thought to be due to fluctuations in light intensity at the time of measurement. When exposed to intense light, photosynthesis is generally a stable process. Conversely, the photosynthesis process experiences a reduction in intensity under low-light conditions due to the equilibrium between the carbon dioxide assimilated during photosynthesis and the amount released during respiration (Lupitasari & Kusumaningtyas, 2020). In addition, variations in photosynthetic rate are also influenced by the tools' accuracy. A plant photosynthetic meter can sometimes be used when the light conditions are slightly dim, but sometimes, it must be used when the light conditions are hot. One leaf sample was measured twice or three times because the tool used could not measure all variables properly, so it was necessary to re-measure to obtain results from the observation variables.

The process of losing water from above-ground plant tissues in the form of water vapor is known as transpiration. This process occurs primarily via the lenticels, cuticle, and stomata; however, it is primarily facilitated by the stomata (Silaen, 2021). There are many factors that affect the transpiration rate, both internal and external. Internal factors refer to characteristics such as leaf count, leaf thickness, leaf surface area, the formation of a waxy coating, density of hairs on the leaf surface, and the quantity of stomatal. External factors are sunlight intensity, temperature, humidity, and wind. The transpiration rate occurs simultaneously with the photosynthesis process when the stomata on the leaves open to take CO_2 and the roots absorb water in the soil, more than 20% of which will be released by plants in the form of water vapor into the air (Anggraini et al., 2021).

The increasing doses of urea fertilizer lead to a higher number of leaves. Urea fertilization causes the availability of nitrogen nutrients in sufficient quantities to produce an environment that supports plant growth and development. The provision of increasingly large doses of urea can increase the nitrogen content in plants so that the accumulation of available nitrogen can be used for the formation of vegetative parts of the plant optimally, especially the formation of leaves (Handayani et al., 2020; Kogoya et al., 2018; Yadav et al., 2022; Zaman et al., 2022). The total overall area of the leaves exhibits a linear relationship with the number of leaves, meaning that it increases in direct proportion to the increase in leaf count. The cause of this phenomenon is the existence of a direct correlation between the number of leaves and the surface area of each leaf. Consequently, this leads to an increased rate of transpiration. This is because a greater leaf area is directly associated with a higher number of stomata on the leaves, which in turn impacts the rate at which plants release water vapor through transpiration. (Maylani et al., 2020). Leaf thickness affects the transpiration rate of plants. Leaf thickness negatively correlates with a transpiration rate, which shows an inverse ratio between leaf thickness and a transpiration rate. The lower the leaf thickness, the higher the transpiration rate. On the other hand, if a plant's leaf is thicker, its transpiration rate will be lower (Dacosta & Daningsih, 2022). The width of stomatal openings is directly proportional to the stomatal conductance and the transpiration rate. The greater width increases conductance and transpiration. The turgor pressure of the guard cells influences the opening and closing of the stomata. In high soil moisture conditions, the turgor pressure of the stomatal guard cells increases, which causes the opening of the stomata so that the stomatal conductance is maximized. Conversely, in low soil moisture conditions, the turgor pressure of the guard cells will decrease, resulting in stomatal closure and a decrease in stomatal conductance. In high soil moisture conditions, the turgor pressure of the stomatal guard cells increases, which causes the opening of the stomata so that the stomatal conductance is maximized. Conversely, in low soil moisture conditions, the guard cell turgor pressure will decrease, resulting in stomatal closure so that stomatal conductance decreases (Boy et al., 2022). The high nitrogen supply will increase stomatal density (Zhu et al., 2020), increasing the plant transpiration rate (Cechin et al., 2022).

Chlorophyll, or leaf green pigment, is vital for plant photosynthesis. Plants have two different kinds of chlorophyll, chlorophyll a, and chlorophyll b, which function like photosynthetic photoreceptors that capture sunlight for synthesizing carbohydrates from CO_2 and water, becoming energy sources during plant growth and development. The photosynthetic ability of each plant varies based on internal and external factors, including the amount of chlorophyll present (Siswanti & Riesty, 2021). This study found that variations in amaranth and urea application had no interaction effect on leaf chlorophyll content ($p>0.05$). The results indicate a notable disparity in leaf chlorophyll content based on the treatments. Red amaranth varieties exhibited significantly ($p<0.05$) greater amounts of chlorophyll a, b, and total chlorophyll retrieved from green varieties. The application of 150 $kg.ha^{-1}$ of urea led to a greater concentration of chlorophyll a, significantly different ($p<0.05$) from

Table 4. Chlorophyll Content of Leaves

Treatment	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total Chlorophyll ($mg.g^{-1}$)
Amaranth			
Green	0.467 a	0.220 a	0.687 a
Red	0.607 b	0.411 b	1.017 b
Urea ($kg.ha^{-1}$)			
0	0.492 ab	0.262 ab	0.754 ab
50	0.415 a	0.194 a	0.609 a
100	0.561 bc	0.318 abc	0.879 bc
150	0.620 c	0.347 bc	0.966 bc
200	0.544 bc	0.306 abc	0.850 bc
250	0.557 bc	0.352 bc	0.908 bc
300	0.571 bc	0.429 c	1.000 c
Interaction	-	-	-

Remarks: Mean values associated with the same letters are not significantly different according to Duncan's Multiple Range Test at 0.05 level of significance.

the treatment retrieved from 50 kg.ha^{-1}. Conversely, the treatment of 300 kg.ha^{-1} treatment yielded the greatest quantity of chlorophyll b and total chlorophyll, both of which were significantly distinct ($p<0.05$) from 50 kg.ha^{-1} treatment (Table 4).

According to Nugroho et al. (2023), the quantity of chlorophyll a, chlorophyll b, and total chlorophyll, which is present within the microgreen of red amaranth, is greater than that within the microgreen of green amaranth. Chlorophyll is crucial for plant development, particularly in terms of the color of red and green amaranth. Red amaranth contains more chlorophyll than green amaranth because it has a greater capacity to protect itself from the harmful effects of light. Red amaranth enhances the ability of photosynthetic organs to protect themselves from damage caused by intense light, both at the level of the photosystem and overall photodegradation. Photoprotection is a process that protects chlorophyll and photosynthetic membranes from damage caused by light. This is accomplished by synthesizing crucial botanical pigments, including anthocyanins, betalains, betacyanins, and carotenoids (Landi et al., 2013). Carotenoids are pigments that aid photosynthesis and protect photosynthetic apparatus. Their concentration increases by up to 115% with higher nitrogen supply compared to low nitrogen conditions, according to Cechin et al. (2022).

In addition, plant age, leaf morphology, and genetics can also affect the amount of chlorophyll in plants. Physiological stages and leaf age affect the chlorophyll content of a plant. Plant species of the same age can contain different chemicals with different genomes, leading to different metabolisms depending on the number of substrates and metabolic enzymes. The leaf area can indicate leaf chlorophyll content. A wider leaf surface indicates more chlorophyll. The greater amount of chlorophyll will increase the photosynthesis process in plants, so production will also increase (Wahyuningtyas et al., 2022; Wijaya et al., 2020). The leaf surface area helps absorb light for photosynthesis in low light conditions. Leaves that have a wide leaf area morphology will be able to capture light optimally. Then, the thickness of the leaf can also affect the amount of chlorophyll in it. Thin leaf morphology usually languishes more when picked, so the decrease in chlorophyll content is easier. The existence of adaptation factors or the dominance of other pigments in the leaves can also cause differences in chlorophyll levels in plants (Dharmadewi, 2020).

Nitrogen is a fundamental element in the chlorophyll molecule, so an increase in nitrogen supply leads to higher chlorophyll concentration in plants (Cechin et al., 2022). In amaranth leaves, chlorophyll is essential for producing nutrients for growth and development. Leaf chlorophyll content increases with nitrogen fertilizer doses (Di Mola et al., 2020). Implementing 300 kg.ha^{-1} of urea led to the highest overall chlorophyll content, while no notable differences were observed among varying urea doses. Plants that lack nitrogen can experience several negative impacts, such as leaves falling off easily, stunted growth, and reduced production. Additionally, plants with low chlorophyll content may struggle to absorb light, decreasing photosynthate production (Utami, 2020).

CONCLUSION

This study examines the physiological responses of amaranth plants to the application of urea fertilizer. The research shows no interaction between amaranth varieties and urea doses regarding plant growth and physiological activity. The physiological response of both amaranth varieties to urea fertilization is the same. In general, adding urea fertilizer stimulates the vegetative growth of amaranth plants, as indicated by the total chlorophyll content.

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AUTHOR'S CONTRIBUTIONS

FCWS conceptualized, conceived and designed the study, performed the experiments, analysed the data, wrote the manuscript, and reviewed and approved the final version. EP conceived and designed the study contributed to the data validation and final review of the manuscript, oversaw the study's conduct, and reviewed and approved the final version. MR conceived and designed the study contributed to the manuscript's data validation and final review, oversaw the study's conduct, and reviewed and approved the final version. ATS conceived and designed the study.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Effects of Water Clover Density and Submerged NPK Fertilizer on Rice Production

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ABSTRACT

Water clover is an important weed that causes a decrease in rice yields by 15-42%. This study examined the effects of water clover density andNPK fertilizer application on rice yields. This study was arranged in a complete randomized block design with two factors. The first factor was water clover density with three levels, consisting of no water clover, one water clover, and two water clover. The second factor was the application of NPK, which was applied to the surface, submerged in 3, 6, and 9 cm—performed with three replications. Water clover density affected the number of rice panicles, the number of rice grains per panicle, the weight of 100 seeds, rice biomass, and water clover biomass. The application of submerged NPK affected rice biomass. The application of NPK on the surface reduced the highest rice biomass. Two water clovers per rice plant reduced the number of rice panicles by 39% compared to without weeds. Rice biomass decreased by 40% and 50% at weed density of one and two water clover weeds per rice plant, respectively. The higher the density of clover weeds, the higher the decline in harvest yields and the higher the biomass of clover weeds.

Keywords: Number of tillers; Rice biomass; Water clover biomass; Weight of 100 seeds

INTRODUCTION

Weeds are a significant problem in Indonesia's rice production. Rice yield losses due to weeds reach 10 to 86% (Simarmata et al., 2023). Yield losses can be reduced by controlling weeds through a cultivation system approach and weed control techniques. Increasing yield loss can cause food problems because rice is Indonesia's leading food crop commodity (Dwipa et al., 2023). The need for rice increases with population growth, in which household consumption 2022 is 20,685,619 tons. Therefore, increasing the productivity of rice plants is always attempted by suppressing weed growth (Cuaton & Delina, 2022). The most common weeds found in rice cultivation are water clover weeds. Water clover weeds are difficult to control, so they can cause suboptimal rice growth and reduce rice yields (Sulaiman et al., 2022). The decrease in rice yield due to water clover weed reached 15-42% for lowland rice and 47-87% for upland rice (Rahaman et al., 2022). Water clover weed has taproots, so the roots reach deep and spread between the rice plants, leading to the competition between weeds and rice in getting water, nutrients, and light (Az-Zahro, 2022). Weeds are competitive over cultivated

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rice since they grow taller and faster (Vu et al., 2023). Therefore, the disturbing presence of weeds is a persistent problem in rice plants (Kang-xu et al., 2022).

Weeds compete for survival with their environment above and below ground (Papapanagiotou & Vasilakoglou, 2023; Zahra et al., 2022). Weed associations may form around the primary crop if the growth requirements of weeds and the crop are similar. These associated weeds will compete for the materials they need, especially if the amount is minimal for both. Competition will be even more intense if the amount of materials competed for cannot be used together (Rodenburg et al., 2022). The highest degree of competition occurs during a critical period of growth. Cultivated plants experience a critical period when they are most sensitive to the environment, especially in competition for space, nutrients, water, and sunlight. If weeds are present and interfere with cultivated plants, cultivated plants cannot compete in utilizing the main growing environmental factors because cultivated plants are at their weakest (critical period) (Awan et al., 2022).

In addition to the weed problem, fertilizer is another important factor in improving rice yield and quality. One type of inorganic fertilizer that is effective in raising the availability of macronutrients (potassium, phosphate, and nitrogen) is compound fertilizer (Haouas et al., 2021). Compound fertilizer has a higher effectiveness in providing nutrients compared to single fertilizers. Compound fertilizers significantly stimulate plant growth and yields because nitrogen, phosphate, and potassium contribute nutrients available to plants that can support plant vegetative and generative growth (Choo et al., 2020; Singh et al., 2021). However, the depth of fertilization can be considered due to the root system of rice and weeds. Applying submerged fertilizer can be more effective than mulching because it can reduce the evaporation of organic matter (Duan et al., 2023; He et al., 2023). This study examined the effects of water clover weed density and NPK fertilizer application on rice yields.

MATERIALS AND METHODS

The research was conducted at the Field Laboratory in Bakaran Hamlet, Jumantono District, Karanganyar, Central Java, Indonesia, at coordinates 7°37°51°S 110°56°52°E. The study research was arranged in a factorial, completely randomized block design with two factors. The first factor was water clover density, namely without water clover, one water, and two water clovers with a 10-15 cm height. The second factor was the application of NPK, namely control or applied to the surface, NPK submerged in 3 cm, NPK submerged in 6 cm, and NPK submerged in 9 cm. Each treatment combination consisted of three replications, resulting in 36 experimental units. Each treatment consisted of 30 rice plant populations. The rice planting space used was 20 x 20 cm, with a plot size for each treatment of 1.2 m^2. The study used IR32 rice seeds, water clover weed 10 - 15 cm high, polybag size 50 x 50 cm, NPK fertilizer, organic fertilizer, soil, and water. The soil used in the study was alfisol soil with an acidic pH of 6, shallow organic C content of 0.387 %, external total N content of 0.08 %, moderate total P_2O_5 content of 29.21 mg/100 g, K_2O content, low total of 12.26 mg/100 g, shallow C/N content of 4.84, specific gravity including clay minerals (2.14 g/cm^3), volume weight including mineral soil (1.2 g/cm^3), and moderate porosity of 43.93. The tools used were trowels, buckets, sprayers, an oven at 60 °C, and measurement tools, including meters, rulers, and digital scales. The observed yield variables included the number of rice tillers, number of panicles per hill, number of seeds per panicle, weight of 100 seeds, rice biomass, and clover biomass. All yield variables were observed at 42 days after planting. Data were analyzed using an analysis of variance of 5 %. If it was significant, the test of significant differences between treatments was continued with the 5 % Duncan Multiple Range Test.

RESULTS AND DISCUSSION

The density of water clover weeds and submerged NPK application did not affect the number of rice tillers (Table 1). The higher density of water clover weeds can cause a decrease in the number of rice tillers. The density of one weed led to a decline of 17.40%, while the density of two weeds caused a 22% fall compared to non-weeds. The tiller decrease was due to competition between rice and water clover weeds. Weeds can compete in absorbing water and nutrients (Li et al., 2022). The number of rice tillers correlates with the number of rice panicles, so the higher the number of tillers, the higher the number of rice panicles. Water clover weed density significantly affected the number of rice panicles (Table 2). The density of the two water clover weeds showed the lowest number of rice panicles, which was 5.17. The number of rice panicles at the density of one weed decreased by 26%. Meanwhile, the density of two weeds decreased the number of panicles by 39%. The combination of NPK fertilizer submerged in 3 cm with one weed density produced the lowest number of tillers, which was 1.77. This shows that there is competition between weeds and rice. Weed population density determines competition, leading to a decrease in crop production (Maciel et al., 2022). In addition, weeds that appear earlier or simultaneously with cultivated plants significantly impact plant growth and yields (Monteiro & Santos, 2022). Weed competition at the start of growth will

Table 1. Effects of the water clover density and application of NPK on the number of rice tillers per plant

Water clover density per rice plant	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	2.77	3.22	3.00	3.66	3.16
1	2.89	1.77	2.55	2.78	2.50
2	2.00	2.55	3.11	2.78	2.61
Average	2.55	2.51	2.89	3.07	-

Remarks: (-) there is no interaction between treatments

Table 2. Effects of the water clover density and application of NPK on the number of rice panicles per plant

Water clover density per rice plant	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	7.33	8.33	8.67	9.67	8.50b
1	4.67	7.33	7.33	5.67	6.25a
2	4.00	5.33	6.00	5.33	5.17a
Average	5.33	7.00	7.33	6.89	-

Remarks: Means followed by the same letters in the same column are not significantly different, (-) there is no interaction between treatments

Table 3. Effects of the water clover density and application of NPK on the number of spikelets per rice panicle

Water clover density	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	50.33	46.33	53.33	48.78	49.69b
1	39.00	42.33	46.44	44.89	43.17a
2	35.55	42.00	37.80	38.22	38.39a
Average	41.63	43.55	45.86	43.96	-

Remarks: Means followed by the same letters in the same column are not significantly different, (-) there is no interaction between treatments

Table 4. Effects of the water clover density and application of NPK on the weight of 100 seeds

Water clover density	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	2.27	2.34	2.58	2.36	2.39b
1	2.18	2.23	2.36	2.12	2.22ab
2	1.97	2.13	2.09	2.05	2.06a
Average	2.14	2.23	2.34	2.18	-

Remarks: Means followed by the same letters in the same column are not significantly different, (-) there is no interaction between treatments

reduce yield quantity, while competition and weed disturbance before harvest majorly affect yield quality (Grzanka et al., 2022).

The results showed that the density of weeds affected the number of shoots per rice panicle (Table 3). The highest number of nodes per panicle was in the treatment without weed. The number of rice grains decreased with the higher density of weeds. The rice grains were reduced by 22% in the treatment of two weeds per rice plant compared to without weeds. This indicates competition for water, nutrients, and light (MacLaren et al., 2020). According to Dass et al. (2017), weeds require between 330 and 1900 liters of water for every kilogram of organic matter, which is nearly twice as much as plants do. Furthermore, because weeds' roots are deeper and wider than rice's, weeds' water clovers absorb nutrients at a quicker rate than rice plants (Antralina, 2012). Weeds and rice also experience competition for light. Faster-growing weeds can shade rice plants so that the light received by rice plants is reduced. This decrease in sunlight can cause a reduction in the rate of photosynthesis so that plant growth and yield are not optimal (Rogowski et al., 2019). The lowest number of panicles was in the combination of NPK applied to the soil surface with the treatment of two weeds per rice plant, which was 35.55 panicles. These results indicate that fertilizer application to the soil surface causes leaching, and the nutrients cannot be easily absorbed by plants.

Water clover density affected the weight of 100 seeds (Table 4). However, the application of NPK did not affect the weight of 100 seeds—the highest 100-seed weight was found in the treatment without weeds. The weight of 100 seeds at the density of one weed per rice plant was not significantly different from the treatment without weeds. However, the density of two weeds per rice plant caused a higher reduction in the weight of 100 seeds. The weight of 100 seeds decreased due to competi-

Table 5. Effects of the water clover density and application of NPK on the rice biomass (g)

Water clover density	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	14.84	14.63	17.14	15.35	15.49b
1	5.32	11.80	10.70	8.85	9.17a
2	5.43	7.29	7.36	10.43	7.63a
Average	8.53a	11.24b	11.74b	11.55b	-

Remarks: Means followed by the same letters in the same column are not significantly different, (-) there is no interaction between treatments

Table 6. Effects of the water clover density and application of NPK on the water clover weed biomass

Water clover Density	NPK Fertilizer Application				Average
	In Surface	Submerged in 3 cm	Submerged in 6 cm	Submerged in 9 cm	
0	0.00	0.00	0.00	0.00	0.00a
1	20.00	18.33	6.33	19.00	15.92b
2	35.33	46.00	43.33	21.00	36.42c
Average	18.44	21.44	16.56	13.33	-

Remarks: Means followed by the same letters in the same column are not significantly different, (-) there is no interaction between treatments

tion between weeds and rice plants. The negative effect of weeds is that they reduce yield rates due to competition in taking nutrients, water, and light (Beiermann et al., 2022). Thus, to replace the competition, fertilization is carried out correctly. Fertilization aims to provide additional nutrients to the soil, which plants directly or indirectly absorb (Ma et al., 2021).

Water clover weed density and NPK application significantly affected rice biomass (Table 5). Without weed density, the highest rice biomass was 15.49 g. The density of one or two weeds per rice plant caused a decrease in rice biomass. Rice biomass decreased progressively with the increasing weed density because weed density could increase competition between weeds and plants. In addition, this water clover weed has secondary metabolites that can play a role in inhibiting the growth of rice plants (Zhu et al., 2021). Efforts to support rice yields are carried out by fertilizing with NPK fertilizer. However, this fertilizer application must be done correctly. The results showed that the application of NPK fertilizer submerged in 6 cm showed the highest rice yield because rice has fibrous roots, making the root reach not deep and wide. The soil's physical state influences rice roots' growth (Wu et al., 2020; Xiong et al., 2021). A dense soil structure will inhibit the rate of deeper root penetration. NPK fertilizer can affect plant length growth, increasing meristem activity yield at the growing point. The higher the action, the higher the plant biomass,

Water clover weed density significantly affected water clover weed biomass (Table 6). The highest water clover weed biomass was observed at the density of two weeds per rice plant, which was 36.42 g. This shows that the higher density indicates faster weed growth. The water clover biomass ratio was higher than rice biomass. This shows that water clover weed can absorb more water and nutrients than rice (Guntoro & Fitri, 2013; Kumawat et al., 2022; Vu et al., 2023). This increased absorption is supported by the characteristics of the water clover root, namely the taproot, so the roots are not deep and broad. The wider these roots reach, the more water absorption and nutrients can be increased.

CONCLUSION

Application of NPK fertilizer submerged in the soil could increase the number of rice tillers, the number of rice panicles, and rice biomass. Two water clover weeds per rice plant reduced the number of rice panicles by 39% compared to without weeds. Rice biomass decreased by 40% at one weed density per rice plant and 50% at the weed density of two weeds per rice plant. The higher water clover weed density caused the highest yield decrease. In addition, the higher the weed density, the higher the water clover weed biomass.

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AUTHOR'S CONTRIBUTIONS

MTSB was instrumental in designing and supervising the entire study. S was involved in data analysis and preparation of the research results. MR contributed to data collection and validation processes. DS was responsible for writing the initial draft of the manuscript and editing. LIS contributed to the literature review and final revision of the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Goatweed Flower Extract (*Ageratum conyzoides* L.) as a Botanical Insecticide for Pest Control *Crocidolomia binotalis* Z.

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ABSTRACT

Crocidolomia binotalis Z. is an important cabbage plant pest in Indonesia. Nevertheless, pest control strategies depend on synthetic pesticides, negatively impacting ecosystems. Therefore, developing an effective organic pesticide approach to controlling *C. binotalis* is necessary. This research aimed to test the effectiveness of goatweed flower extract (*Ageratum conyzoides* L.) in controlling pest *C. binotalis*. The research consisted of two sets of experiments with two methods: the stomach and contact poison. Each experimental set was arranged in a completely randomized design (CRD) with three replications. The treatments were six levels of extract concentration at 0%, 15%, 30%, 45%, 60%, and 75%. The results showed that the application of *A. conyzoides* flower extract using the stomach and contact poison methods significantly increased the percentage of larval mortality 24 hours after application (haa) and the percentage of total larval mortality. The flower extract treatment of *A. conyzoides* significantly reduced the percentage of leaf area eaten, increased larval mortality, inhibited pupation and imago emergency, and shortened the larval stage's duration. The percentage of larval mortality through contact poison was higher than stomach poison.

Keywords: *Ageratum conyzoides*; Botanical Insecticide; *Crocidolomia binotalis*; Effectiveness

INTRODUCTION

Pests and pathogens are a major limiting factor for production in agricultural cultivation. One such pest is *Crocidolomia binotalis* Z., which poses a significant threat to cabbage plants in Indonesia (Fifi et al., 2022; Tarigan et al., 2021). The damage caused by this pest can significantly reduce the quantity and quality of cabbage production, with agricultural losses reaching almost 100% by *Plutella xylostella* (Fifi et al., 2022; Barita et al., 2018). Currently, synthetic insecticides are the primary method of managing these two pests. Farmers' dependence on synthetic insecticides encourages excessive use of these chemicals (Nurhudiman et al., 2018; Tampubolon et al., 2018). The long-term application of chemical insecticides results in various negative impacts, such as environmental pollution, the eruption of secondary pests, disruption of human health, pest resistance, and resurgence (Wahyudin et al., 2021). Therefore, alternatives or supplements to synthetic pesticides should be explored to mitigate the negative impacts they can cause (Nurhudiman et al., 2018; Tampubolon et al., 2018).

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In recent years, efforts have been made toward obtaining safer alternatives to chemical insecticides for sustainable pest management. Habitat manipulation, a part of conservation biological control, aims to provide floral resources, alternative prey, and shelter to predators and parasitoids to enhance and sustain natural pest suppression. The use of plant extracts as botanical insecticides is also an important provisioning of ecosystem service (Amoabeng et al., 2019). Botanical insecticides contain active ingredients derived from secondary compounds produced by plants. The toxicity of these secondary compounds has various effects on insect behavior or physiology. Some secondary compounds can interfere with the growth and development of immature insects, affect feeding behavior, reduce fecundity, and shorten the survival of insect imago (Sultan et al., 2016; Nurhudiman et al., 2018; Tampubolon et al., 2018).

Among the thousands of plant species studied, goatweed (*Ageratum conyzoides*) is one species that contains bioactive compounds with insecticidal properties (Tampubolon et al., 2018; Katuuk ., 2019; Amoabeng et al., 2019). This plant contains *antijuvenile hormone*, precocene compounds, coumarins, eugenol, HCN, alkaloids, and steroids in the roots, leaves, and flowers (Tampubolon et al., 2018). These toxic compounds can affect the respiration process, inhibit seed growth, and inhibit microbial activity (Cahyati & Sutanto, 2021). Sharifi-Rad et al. (2021) stated that coumarin could convert into dicoumarol, which is a blood anticoagulant and causes death. Several previous studies have shown that goatweed extract can control aphids (*Aulocaphora* sp.) on cucumber (*Cucumis sativus*), where a concentration of 9 % can interfere with their eating ability, as evidenced by the decrease in aphid appetite by up to 20 % and cause a lethargic reaction in aphids, which ends in their gradual death (Sultan et al., 2016). Goatweed extract contains alkaloids; this compound causes pests/insects to lose their appetite and die. Alkaloids can also inhibit insect growth, leading to metamorphosis failure (Dewi, 2016). Meanwhile, concentrations between 1-5 % were able to cause mortality in *Plutella xylostella* larvae (Nurhudiman et al. 2018).

Goatweed can be utilized as a biopesticide because the active ingredients in the leaves can inhibit the development of insecticides either directly or indirectly. However, research investigating the most effective use of this extract is still limited. According to Wahyudin et al. (2021), the mechanisms by which insecticides work can be divided into (1) Based on how they enter the body of insects, they can be categorized into digestive or gastric poisons, contact poisons, neurotoxins, protoplasmic toxins, and systemic poisons; and (2) Based on the chemical composition, insecticides are classified into organochlorines, organophosphates, carbamates, and pyrethroids. Based on the background, this research was conducted to determine the effectiveness of goatweed flower extract (*Ageratum conyzoides* L.) in controlling the pest *Crocidolomia binotalis* Z. The use of the stomach poison method and the contact poison method with different concentration levels of goatweed extract aimed to determine the effectiveness of the chemical content of goatweed as a pesticide against *C. binotalis* Z.

METHODS

Experimental Design

The research was conducted for four months at the Arthropoda Pest Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Lampung. This study consisted of two sets of tests; the first was to examine the effectiveness of goatweed flower extract (*A. conyzoides*) against *C. binotalis* pests using the stomach poison method, and the second was to determine the effects of the contact poison method on *C. binotalis* pests. The research was arranged in a completely randomized design (CRD) with three repetitions and six treatments. The treatments consisted of six concentration levels determined based on the results of preliminary tests, namely 0 %, 15 %, 30 %, 45 %, 60 %, and 75 %. Thus, each research set consisted of 18 research units. In each research unit, 20 third-instar *C. binotalis* larvae were invested.

Preparation and Application of Flower Extract of *Ageratum conyzoides* L.

A. conyzoides flowers were washed and dried indoors for a week. Then, the flowers were ground into powder. 100 g of powder was mixed with 100 ml of solvent (distilled water) and blended until smooth and filtered. As an emulsifier, detergent powder (1 g per liter of water) was used, and to increase the solubility of the powder, acetone (1 %) was added as much as 10 ml per water, and agristic (0.05 %) was added as an adhesive. The resulting extract was a 100 % concentration (aliquot), ready to dilute the extract according to the concentration level to be tested.

Testing of *A. conyzoides* flower extracts was carried out at six concentration levels by diluting the aliquot concentration with solvents (consisting of distilled water, detergent, acetone, and agristic) into test concentrations, namely 0; 15; 30; 45; 60; and 75 ml in 100 ml solution.

Insect breeding test of *C. binotalis* larvae was obtained from cabbage plantations in Gisting Atas Village, Tanggamus Regency. Larvae were obtained, collected, and fed with mustard leaves in an aerated tray with a mixture of soil and sand (1:1 ratio by weight) for pupation. Pupae encased in soil granules were transferred and reared in clear, aerated plastic cages.

Emerging moths were fed with 10% liquid honey (v:v) and continued to be reared until they laid eggs on the prepared mustard leaf pieces. The mustard leaf pieces were transferred to an aerated tray until they hatched. The emerged larvae were reared until sufficient numbers for testing (360 individuals/ experimental set).

Application of Flower Extract on *C. binotalis*

The application stage of *A. conyzoides* flower extract was carried out using the stomach and contact poison methods.

Stomach poison method

The cabbage pieces (7 x 10 cm) were dipped in the test extract for five minutes; the excess liquid was absorbed with tissue paper, then removed with tweezers and aerated for five minutes. Control leaves were dipped in distilled water containing adhesives, acetone, and emulsifiers. The application was made by placing 20 of the 3rd instar of *C. binotalis* larvae in a plastic jar (diameter of 11.5 cm and height of 11.5 cm) and allowing them to feed on the treatment or control leaves for 24 hours. Afterward, the jars were cleaned, and the larvae were fed with untreated leaves. The test insects were observed every day until they became imago.

Contact poison method

The larvae of *C. binotalis* in each experimental set were placed in a petri dish. Then, the extract test was applied through spraying according to the concentration level of the extract tested. The extracted liquid was sprayed on the entire body of the larvae (4 sprays or 4 ml). The larvae were placed in plastic jars and fed with untreated cabbage leaves. The insects were kept for observation every day until they became imago.

Observation

The observed and calculated variables were: 1) Percentage of larval mortality 24 hours after application; 2) Percentage of leaf area consumed; 3) Total larval mortality, which is the percentage of the total number of larvae that died after all the test insects had pupated); 4) Larval lifetime; 5) Percentage of formed pupa; 6) Time to pupation; 7) Percentage of imago emergence; and 8) Duration of imago life.

The leaf area consumed by larvae was explicitly observed when applying *A. conyzoides* flower extract via the stomach poison method. Observations were made 24 hours a day by measuring the leaf area consumed by the larvae (cm^2) using a leaf area meter type LI-300 (Li-cor). The leaf area provided for feeding was 140 cm^2.

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA). Furthermore, the data were processed with the least significant difference test (LSD) at a significant level of 1 % or 5 % to determine the difference in the mean value between treatments. A probity analysis was conducted to determine the LC 50 value, especially for larva mortality data.

RESULTS AND DISCUSSION

Percentage of Larval Mortality

The results showed that the application of *A. conyzoides* flower extract using the stomach and contact poison methods significantly increased the percentage of larval mortality 24 hours after application (haa) and the percentage of total larval mortality. The results of the stomach poison method after immersion with *A. conyzoides* flower extract at a concentration of 15 % to 75 % were significantly higher than at the concentration of 0 % (control). The percentage of larval mortality at 24 haa was the highest at a concentration of 60 % and 75 %, which was 23.33 %, while the lowest was at a concentration of 15 %, which was 10 %. At a concentration of 15 % extract, larval mortality was not significantly different from those at concentrations of 30 % and 45 % but was significantly lower than those at concentrations of 60 % and 75 %. It can be observed that the percentage of larval mortality 24 haa did not experience a significant increase at the concentration level of 30 % to 75 % (Figure 1).

Similarly, with the contact poison method, the percentage of larval mortality of *C. binotalis* 24 has increased with the increasing concentration of *A. conyzoides* flower extract. The highest percentage of larval mortality at 24 haa was 38.33 % at a 75 % extract concentration.

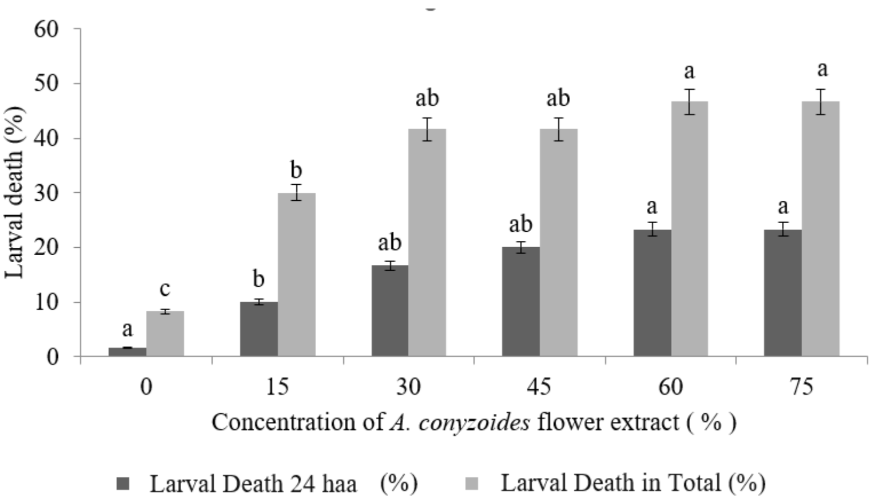

Figure 1. Effects of *A. conyzoides flower extract* concentration treatment through the stomach poison method on the percentage of larval mortality 24 haa. and the percentage of total larval mortality. Bars associated with the same letters are not significantly different at the 5% significance level according to the LSD test.

Still, the increase in the percentage of mortality was not significantly different from those at the concentrations of 30 %, 45 %, and 60 %. The percentage of larval mortality 24 hours after application at a concentration of 15% was significantly higher than that at 0 % concentration (control) but lower than the other treatments (Figure 2). Based on the findings, it is known that the effectiveness of the poison contact method in killing larvae is approximately 15 % better than the stomach poison method.

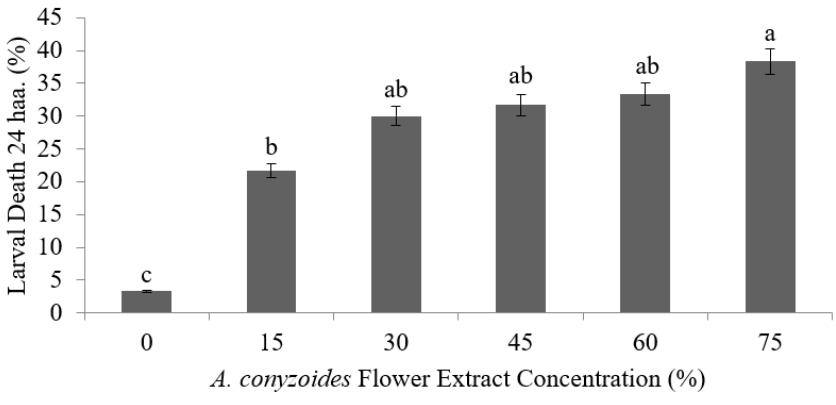

Figure 2. Effects of *A. conyzoides flower extract* concentration treatment through the contact poison method on the percentage of larval mortality 24 haa. Bars associated with the same letters are not significantly different at the 5% significance level according to the LSD test.

The percentage of total larval mortality of *C. binotalis* due to the effect of *A. conyzoides* flower extract treatment through the stomach poison method was relatively low, which was 46.6 7%, so no probity analysis was carried out to determine the LC_{50} value of *A. conyzoides* flower extract treatment. The highest percentage of total larval mortality occurred at the concentration level of 60 % and 75 %, which was 46.67 %. Still, the treatment effect at the two concentrations was not significantly different compared to the concentrations of *A. conyzoides* flower extract of 30 % and

45 %. However, the total larval mortality at a concentration of 15 % was significantly lower than at concentrations of 60% and 75 %. The 15 %-75 % extract concentration was significantly higher than the control (0% concentration). Still, there was no significant increase in the percentage of total larval mortality at concentrations of 30 %, 45 %, 60 %, and 75 % (Figure 1). Slightly different from the stomach poison method, the contact poison method significantly increased the percentage of total larval mortality. The results showed that the percentage of total larval mortality due to the effect of *A. conyzoides* flower extract through the contact poison method exceeded the value of 50 %, which was 60 %. Therefore, probity analysis was conducted to determine the LC_{50} value of *A. conyzoides* flower extract. Based on the results of probity analysis, the concentration level that can cause 50 % of larval mortality is 51.69 %. In addition, a regression equation was obtained, which showed the relationship between the concentration of the extract and the percentage of larval mortality, namely: $Y = 4.05 + 0.56 X$. This means that every 1 % increase in the concentration of *A. conyzoides* flower extract can increase larval mortality by 0.56 %. Without applying *A. conyzoides* flower extract, the larval mortality occurred at 4.05 %.

The stomach poison method, which can cause the death of *C. binotalis* larvae, is thought to be due to the entry of toxic chemical compounds into the insect's body through the digestive system. According to Triharso (1994), toxins enter the body of insects through the digestive system or when ingested, which is closely related to the digestive process. Toxins that enter the mouth are broken down with food by digestive enzymes (salivary enzymes). The entry of these toxins can cause damage to the intestinal tract. In addition, stomach poison that enters the insect's body can be absorbed into the blood and attack the nervous system, causing paralysis (paralysis) and ultimately death. Oguh et al. (2019) also reported that natural pesticides usually target specific sites in the insect, such as the nervous system, resulting in knock-down, lack of coordination, paralysis, and death.

Compared with the stomach poison method, the effect of *A. conyzoides* flower extract treatment through the contact poison method caused a higher percentage of larval mortality in both 24 haa and total larval mortality. This is presumably because the HCN compound in the flower extract of *A. conyzoides* works faster in the insect's body when applied by the contact poison method than the stomach poison method. As Muhidin et al. (2020) reported, HCN compounds are toxic compounds that interfere with the respiratory system. Through the contact poison method, the compound can directly enter the insect's respiratory apparatus through a series of handle holes along the sides of the insect's body (spiracles) (Stejskal et al., 2021). After entering the respiratory system, these toxic compounds affect the central nervous system and can cause rapid paralysis, leading to death. There is much evidence of rapid death from contact poisons, which is 10 minutes after application (Triharso, 1994).

Meanwhile, HCN is less effective when applied by the stomach poison method. This is probably due to the volatile nature of these compounds, which interfere with the respiratory system. Due to its volatile nature, it is possible that the concentration of HCN decreased when the feed was air-dried (for 5 minutes) after being treated with *A. conyzoides* flower extract. Another factor that causes the HCN compound to be less effective is that the poison does not directly hit the target, such as the insect's respiration apparatus. This is different when applied through the contact poison method; HCN compounds can directly hit the insect's respiration apparatus (spiracles) along the sides of the body.

Besides, alkaloid compounds contained in the flowers of *A. conyzoides* specifically interfere with the nervous system of insects (Wahyuni & Loren, 2015). Alkaloids in plants exhibit antibiosis to organisms that ingest them (Metcalf & Luckmann, 1994). Thus, the alkaloid compounds work more effectively through the stomach poison method. However, the possibility of entering the insect's body still exists because application through the contact poison method can affect the insect's mouthparts, allowing alkaloid compounds to enter through the mouth when the insect feeds on host plants. According to Wahyuni & Loren (2015), if the larvae used as test insects die, it may not be solely due to contact with the insecticide but also from ingesting the insecticide. These factors contribute to a higher percentage of larval mortality through the contact poison method than the stomach poison method.

Larval, Pupal, and Imago Stages Duration

The results indicated that the application of *A. conyzoides* flower extract using the stomach poison method significantly shortened the duration of the larval stage but did not significantly affect the pupa and imago stages. On the other hand, the contact poison method had a quietly significant effect on the duration of the larval stage but not on the pupal stage. Furthermore, increasing the concentration of *A. conyzoides* flower extract did not result in different effects on the duration of the larval, pupa, and imago stages when using the stomach poison method (Table 1). The longest larval stage was observed at an extract concentration of 15% to 45%, lasting for 4 days, while the shortest larval stage occurred at a concentration of 75%, lasting for 3.33 days. The data indicated a significant effect of *A. conyzoides* flower extract at concentrations of 15% and 75%, while concentrations of 15%, 30%, 45%, and 60% showed no significant effect (Table 1).

Table 1. Effects of *A. conyzoides* flower extract concentration treatment through the stomach and contact poison methods on the duration of the larval, pupa, and imago stages of *Crocidolomia binotalis*

Concentration (%)	Duration of stage (days)					
	Stomach Poison Method			**Contact Poison Method**		
	Larvae	Pupae	Imago	Larvae	Pupae	Imago
0	5.00 a	8.00	10.33	5.00 a	8.67	8.67
15	4.00 b	8.33	10.33	3.67 b	9.00	8.67
30	4.00 b	8.00	9.33	3.00 bc	8.67	8.67
45	4.00 bc	8.00	10.33	2.67 c	9.67	8.00
60	3.67 bc	8.33	10.00	2.67 c	9.67	6.67
75	3.33 c	8.33	9.67	2.33 c	9.00	7.33
F-count	7.41 **	0.60ns	0.87ns	10.53 **	1.60ns	1.11ns

Note:
** = Different at 1% level of significance
ns = Not significantly different at 1% significance level
Values followed by the same letters in the same column are not significantly different at the 5% significance level according to the LSD test.

In contrast, the contact poison method did not result in different effects on the duration of the larval and pupal stages across concentrations of 15% to 75% (Table 2). Generally, *A. conyzoides* flower extract shortened the larval stage duration as the concentration of the extract increased, influenced by the increasing percentage of larval mortality with higher extract concentration. The shortest larval stage duration was observed at a concentration of 75%, lasting for 2.33 days. However, the reduction in the larval stage duration at these concentrations was not significantly different from concentrations of 45% and 60%. Meanwhile, it was significantly shorter than the larval stage duration at a 15% concentration (Table 2).

Table 2. Effects of *A. conyzoides* flower extract concentration treatment through the stomach and contact poison methods on the percentage of formed pupa and emergence of *Crocidolomia binotalis* imago

Concentration (%)	Duration of stage (days)			
	Stomach Poison Method		**Contact Poison Method**	
	Formed pupa (%)	Appearance of imago (%)	Formed pupa (%)	Appearance of imago (%)
0	91.67 a	85.00 a	90.00 a	78.33 a
15	70.00 b	61.67 ab	55.00 b	46.67 b
30	58.33 bc	50.00 b	50.00 b	43.33 bc
45	58.33 bc	50.00 b	46.67 b	38.33 bc
60	53.33 c	45.00 b	45.00 b	35.00 bc
75	53.33 c	46.67 b	40.00 b	26.67 c
F-count	9.44 **	4.23 *	5.78 **	5.59 **

Note:
** = Different at 1% level of significance
* = Different at 5% significance level
Values followed by the same letters in the same column are not significantly different at the 5% significance level according to the LSD test.

The treatment with *A. conyzoides* flower extract did not significantly affect the duration of the pupal and imago stages. This may be because larvae that successfully formed pupae and emerged as normal imago were relatively resistant to the extract's effects, resulting in unaffected duration of the pupal and imago stages. Nevertheless, the contact poison method was one day more effective than the stomach poison method in reducing the lifespan of insects during the larval stage.

In general, the application of goat weed extract can disrupt physiological processes and induce changes in behavior due to tannin and saponin compounds, which affect the hormonal and nervous systems (Hikmah, 2018). Larvae mature faster but are weak and highly sensitive to physical and chemical pressure, with some failing to pupate and develop into adults (Ngatimin & Uslinawaty, 2019). The contact poison method is purportedly more effective than the stomach poison method in reducing lifespan, allegedly because the contact poison method can reach more bodily systems, such as the respiratory system through inhalation or the circulatory system through the body wall, compared to the stomach poison method, which primarily affects the digestive tract. This aligns with research on *Annona squamosa* L. extract, which was reported to be more effective as a contact poison than stomach poison (Dadang et al., 2011).

The factor causing the duration of the larval stage at a concentration level of 15% to 75 to be significantly shorter when compared to the control is the percentage of larval mortality. The higher the larval mortality percentage, the shorter the larval stage. According to Metcalf & Luckman (1994), the alkaloids contained in plants can allow these plants to have antibiosis properties against organisms that eat them. Antibiotic properties of alkaloid compounds may also be a factor that shortens the larval stage. Based on Table 1, the effect of flower extract treatment of *A. conyzoides* on the duration of pupal and imago stages at concentrations of 15% to 75% was not significantly different compared to the control (0% concentration). This is presumably because the larvae that successfully formed pupae and emerged as normal imago were relatively resistant to the influence of *A. conyzoides* flower extract.

Pupation and Imago Emergence

The results of the application of *A. conyzoides* flower extract using the stomach poison method and contact poison method showed that the treatment of various concentration levels of the flower extract could inhibit the percentage of formed pupa and imago emergence. It was observed that increasing the concentration of flower extract from 15% to 75% did not have a different effect on the percentage of formed pupa and imago emergence. This study revealed that the highest percentage of formed pupa due to the impact of flower extract treatment of *A. conyzoides* was found at an extract concentration of 15%, which was 70.00%, while the lowest percentage of pupae formation occurred at concentrations of 60% and 75%, which was 53.33%. When comparing the application methods, the highest percentage of formed pupa was produced by the extract concentration of 15%, which was 55.00%. In comparison, the lowest percentage of formed pupa was observed at concentrations of 75%, which was 40.00%.

The percentage of larval mortality influenced the decrease in the percentage of imago emergence, but it was also caused by the failure of the defective pupae to emerge as imago. This indicates an inhibition of development from the pupal to the imago stage. According to Vats et al. (2019), this development inhibition is due to *A. conyzoides* plants containing antijuvenile hormone, which inhibits insect development. Additionally, the same result was reported by Poerwanto et al. (2020), who stated that the effect of methanol and ethanol extract of *A. conyzoides* caused inhibition in the pupa phase of *Culex quinquefasciatus* mosquito and larval phase until death. Therefore, it is strongly suspected that this plant's methanol and ethanol extracts have the potential to be larvicidal.

This result suggests that the higher the *A. conyzoides* flower extract concentration, the greater the inhibition of pupation and imago emergence. Likewise, the contact poison method is more effective in inhibiting the pupation and imago emergence than the stomach poison method. This occurs because the toxic compounds that enter the insect's body through the contact poison method disrupt the central nervous system and result in the death of the larvae. In addition, the presence of antijuvenile hormone in *A. conyzoides* (Vats et al. 2019) is thought to be a factor that inhibits the formation of pupae and the emergence of imago.

Percentage Leaf Area Consumed by *C. binotalis*

The results showed that, with increasing concentrations of *A. conyzoides* flower extract, the percentage of leaf area eaten by larvae was lower. However, the increase in extract concentration did not show a consistent pattern to the decrease in the percentage of leaf area consumed by larvae (Figure 3). The highest percentage of leaf area consumed by larvae due to the effect of flower extract treatment of *A. conyzoides* was at a concentration of 75%, which was 60.13%. At concentrations of 15%, 30%, and 45%, the effect of the flower extract of *A. conyzoides* was not significantly different from the

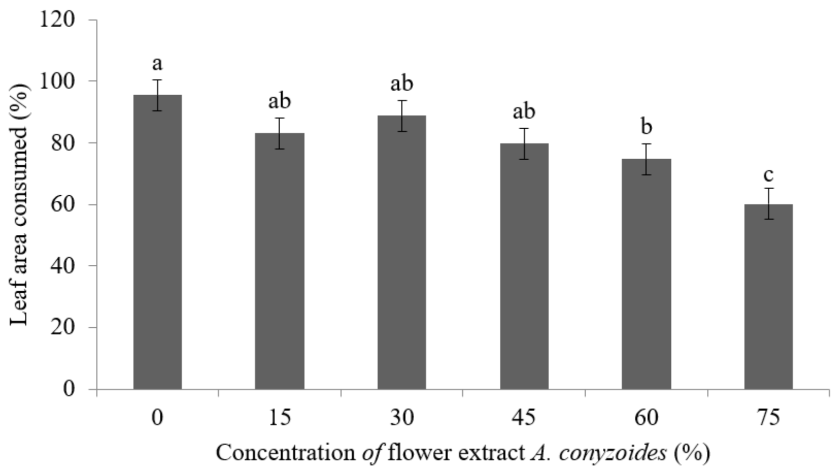

Figure 3. Effects of *A. conyzoides flower extract* concentration treatment through poison method stomach on the percentage of leaf area consumed by *Crocidolomia binotalis* larvae. Bars associated with the same letters are not significantly different at the 5% significance level according to the LSD test.

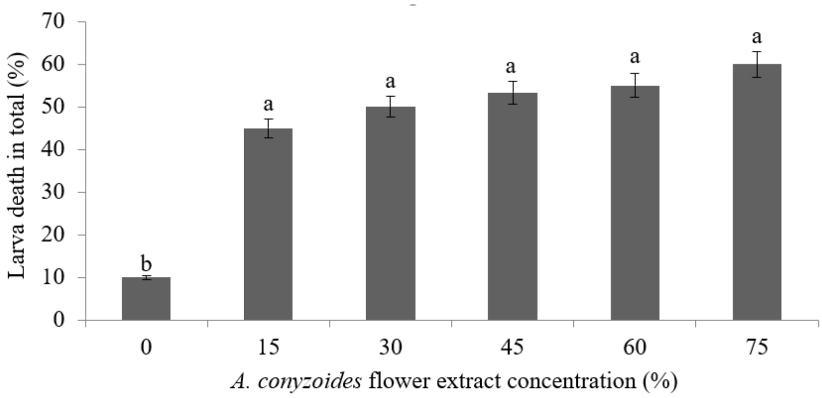

Figure 4. Effects of *A. conyzoides* flower extract treatment through contact poison method on the percentage of total larva mortality. Bars associated with the same letters are not significantly different at the 5% significance level according to the LSD test.

control (0% concentration) (Figure 4). However, there was a significant decrease in the percentage of leaf area consumed by larvae at concentrations of 60% and 75% compared to the control (Figure 3).

The percentage of edible leaf area can be used as an additional parameter in the stomach poison method because the decrease in leaf area consumed by *C. binotalis* larvae indicates that the flower extract of *A. conyzoides* has antifeedant properties against insects. This may be due to coumarin compounds that emit an unpleasant odor and other compounds that cause *A. conyzoides* plants to have a slightly bitter taste for insects (Sultan et al., 2016). Meanwhile, Sari & Armayanti (2018) stated that *A. conyzoides* contain high alkaloids, flavonoids, and tannins, which are concentrated in the leaves. The antifeedant properties against insects might be attributed to alkaloids, as this compound has a bitter taste.

CONCLUSION

Goatweed (*Ageratum conyzoides* L.) flower extract applied through contact poison method was effectively used to control *Crocidolomia binotalis* Z. This can be seen from the results of research showing that administration of goatweed (*Ageratum conyzoides* L.) flower extract significantly increased larval mortality, suppressed percentage of leaf area eaten by larvae, inhibited pupation, suppressed the emergence of imago, and shortened the larval stage duration of *Crocidolomia binotalis* Z.

AUTHOR'S CONTRIBUTIONS

SPN conceptualization, designing, and conducting research. NR research conceptualization. MU analyzes the data, researches it, and writes the result. BS writing and analyzing the research data. BT writing the data research.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Estimating SPAD, Nitrogen Concentration, and Chlorophyll Content in Rice Leaves using Calibrated Smartphone Digital Image

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ABSTRACT

Laboratory analysis is commonly used to determine nitrogen and chlorophyll content. However, smartphones can serve as rapid, mobile, and non-destructive tools for this purpose. An equation can be created to calculate nitrogen and chlorophyll content by analyzing color parameters from digital images of rice leaves. An examination was performed on 86 rice leaf samples from the maximum tillering and mature stages. Rice leaf photos were taken with a smartphone in natural outdoor lighting. Color calibration with Spydercheckr was needed to adjust for lighting conditions. Uncalibrated and calibrated image data were analyzed to determine RGB values converted into CIELAB color space. The L*, a*, and b* values had a significant correlation with SPAD parameters, nitrogen concentration, chlorophyll a, b, and total chlorophyll content. This connection was higher after image calibration. The study found that smartphone images could predict SPAD values with 87.9% to 92.3% precision, depending on color space. Using a smartphone digital picture of L* and a* values, N content could be estimated with 84.7% and 81.9% accuracy. Average accuracy for chlorophyll a, b, and total chlorophyll content was 65% to 76%. This study shows smartphone images can estimate rice leaf SPAD and nitrogen content.

Keywords: Calibrated image; CIELAB; Color image processing, Leaf color; Nitrogen estimation

INTRODUCTION

The development and productivity of plants are predominantly influenced by the physiological mechanisms of photosynthesis and respiration, which facilitate the synthesis of carbohydrates and the generation of energy for plant metabolism. Both metabolic processes can function optimally when provided with the necessary nutrition. Photosynthesis, a biological process affected by chlorophyll in plants, necessitates the presence of nitrogen as a vital component. Hence, the assessment of plant development and production necessitates the consideration of chlorophyll value and nitrogen content in the leaves, as indicated by previous studies (Evans & Clarke, 2019; Mu and Chen, 2020; Stirbet et al., 2020). Prior research has demonstrated the significance of chlorophyll values in the assessment of nitrogen status in leaves, as evidenced by strong correlation values (Gabriel et al., 2019; Zhang et al., 2019).

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Rice is a prominent staple grain globally, with Indonesia being a notable producer of this commodity. At present, Indonesia's food requirements continue to depend on the rice industry, thereby highlighting the significance of government initiatives aimed at enhancing rice yield (Silalahi et al., 2019; Sulistyorini & Sunaryanto, 2020). In order to enhance rice yield, it is important to administer the appropriate dosage of fertilizer, hence enabling the implementation of precision agriculture techniques. The leaf color chart (LCC) is a practical tool designed to assist farmers by providing suggestions on fertilizer application based on color indicators (Subedi et al., 2018). Nevertheless, the utilization of LCC is impeded mostly due to farmers' restricted accessibility.

Furthermore, the qualitative evaluation of color is constrained by using only 4 or 6 shades of green, resulting in an incomplete representation of the entire spectrum of green hues. Moreover, the predominant green hue exhibited by leaves mostly corresponds to the presence of chlorophyll. Consequently, relying just on apparent color characteristics may not be suitable for accurately assessing nitrogen deficit. Furthermore, in order to obtain accurate fertilizer recommendations based on chlorophyll and nitrogen levels, it is necessary to do laboratory analysis, a process that is both costly and time-consuming and not readily available to farmers.

There is a pressing demand for a swift analytical approach that exhibits enhanced accuracy, precision, portability, and universal applicability among wetland rice cultivators. This method should possess the capability of effectively assessing the nitrogen and chlorophyll levels in the leaves, thereby serving as a crucial determinant for determining the appropriate dosage of nitrogen fertilizer to be administered by farmers. The utilization of hyperspectral, multispectral, and infrared cameras, along with other diverse devices, is subject to financial limitations and occasional lack of portability (Caballero et al., 2020; Wang et al., 2021; Wang et al., 2022; Wu et al., 2022). The utilization of widely accessible smartphone cameras has the potential to evaluate the levels of nitrogen and chlorophyll in plants, as the digital images produced by these cameras can offer valuable insights into the color characteristics of leaves. One limitation associated with utilizing digital cameras for color measurement is the susceptibility of the captured color to environmental lighting conditions. Consequently, it becomes imperative to calibrate the color output generated by smartphone cameras.

Variations in color features might arise while capturing digital photographs of an item at different points in time. To address this issue, the calibration of color pixels becomes imperative. The color features obtained can be regarded as standardized colors that can be employed to calibrate color features in smartphone cameras (Cruz, 2019; Souza et al., 2018). Additionally, the Datacolor SpyderCHECKR standard color palette, which serves as a means to calibrate colors and is seamlessly integrated with the SpyderCHECKR software48, is used. Certain studies employ a methodology akin to utilizing the SpyderCHECKR color palette. This involves the placement of multiple calibration objects with distinct colors, enabling the calibration of leaf color features based on the color characteristics generated at various time points. The Spydercheckr color palette is widely utilized in photography as a calibration tool to achieve precise color reproduction. The study conducted by Sunoj et al. (2018) demonstrates that color calibration matrix equations can be constructed using a standard palette of colors. The utilization of digital leaf photos enables the evaluation of plant health by analyzing the colorimetric properties of leaves. The correlation between leaf color and leaf chlorophyll concentration is the most significant, thus indicating that leaf chlorophyll content can serve as a reliable indicator of the overall health status of plants. Therefore, this study aimed to determine the potential of utilizing both calibrated and uncalibrated camera images to estimate chlorophyll and nitrogen content in rice leaves.

MATERIALS AND METHODS

SPAD, Nitrogen and Chlorophyll Content Measurement

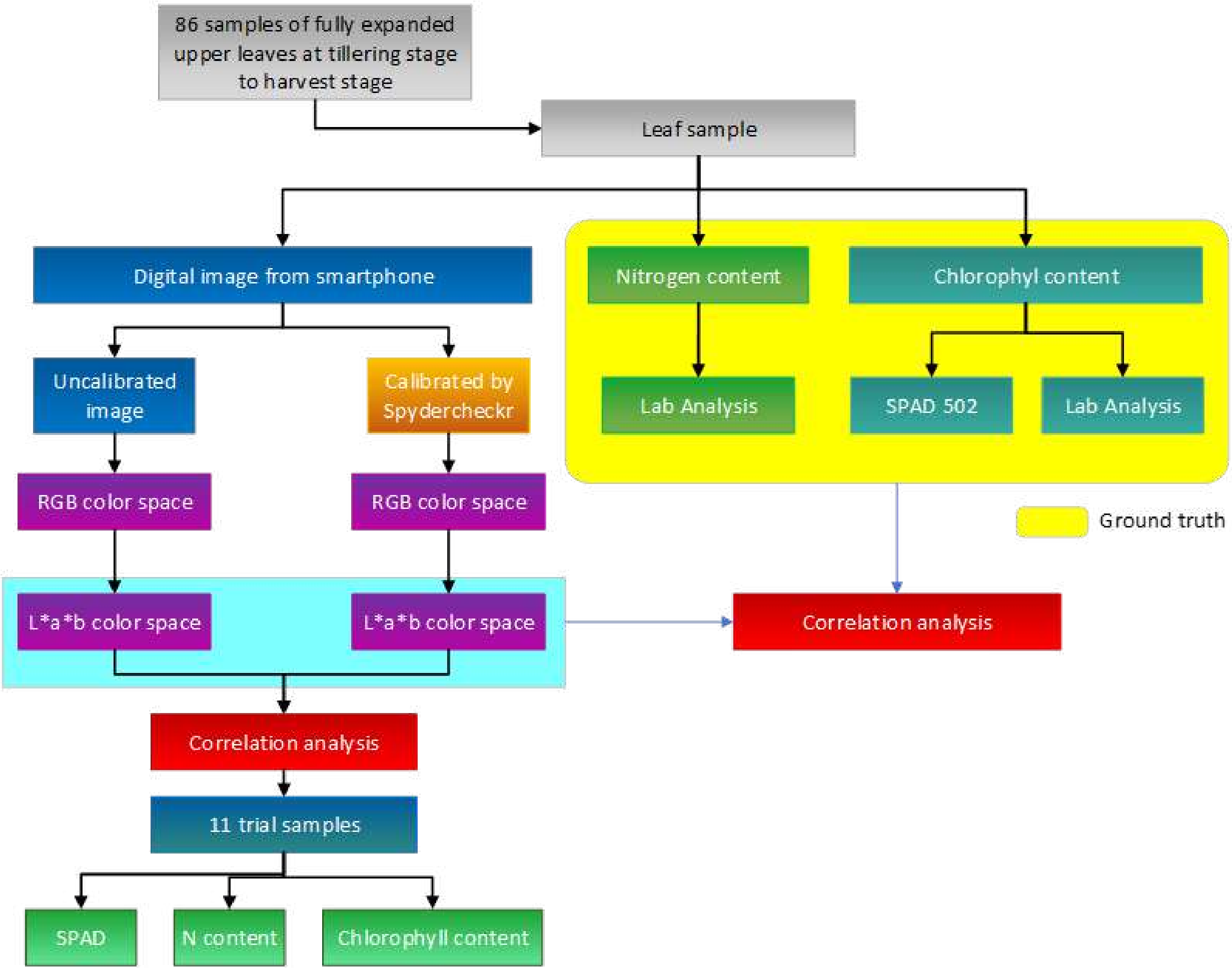

Figure 1. Procedure of leaf sampling and analysis

This study focuses on the relationship between chlorophyll content and leaf N content with image data readings through both calibrated and uncalibrated smartphone cameras. Chlorophyll and leaf N content were measured on fully expanded upper leaves at maximum tillering and harvest stages. A total of 86 plant leaf samples were taken from rice plants of different ages, at maximum tillering period and mature period. Thus, the values obtained were widely distributed. The quantification of chlorophyll content in leaves was carried out using chlorophyll meter and laboratory methods. Leaf chlorophyll content was determined using a SPAD-502 chlorophyll (Konica Minolta Sensing Inc., Tokyo, Japan). This measuring technique involved assessing the transmittance of light at two specific wavelengths at 650 nm (red) and 940 nm (infrared). Chlorophyll content analysis was also measured using the Arnon method in the laboratory. The optical absorption at wavelengths 663 nm and 645 nm was measured using a spectrophotometer device. The instrument was calibrated using a control sample consisting of 80% acetone. The determination of chlorophyll a, chlorophyll b, and total chlorophyll for each sample was conducted utilizing the following formulas:

$$Chl\ a = \frac{(12.7\ x\ D_{663}) - (2.69\ x\ D_{645})\ x\ V}{1000\ x\ W} \quad (1)$$

$$Chl\ b = \frac{(22.9\ x\ D_{645}) - (4.68\ x\ D_{663})\ x\ V}{1000\ x\ W} \quad (2)$$

$$Chl\ total = \frac{(20.2\ x\ D_{645}) + (8.02\ x\ D_{663})\ x\ V}{1000\ x\ W} \quad (3)$$

The variables "V," "W," and "D" represent the volume of acetone 80% (20 ml or 25 ml), the fresh weight, and the optical absorption at wavelengths 663 nm and 645 nm, respectively. The flow of the research method is shown in Figure 1.

Image Acquisition and Processing

Both nitrogen content analysis, chlorophyll measurement using a spectrophotometer, and chlorophyll measurement using SPAD 502 are considered ground truth values that will be correlated with image data from both calibrated and uncalibrated cameras. Subsequent to the removal of the leaves, a digital image of the external environment was conducted employing a smartphone camera equipped with the subsequent specifications and settings (Table 1).

Table 1. Smartphone camera specifications and settings for obtaining digital image data

Specifications			Settings		
Brand	:	Vivo	ISO	:	100
Model	:	V23e	Shutter speed	:	1/400
Resolution	:	64MP	White balance	:	Sunshine
Aperture	:	**f/1.8**	Autofocus	:	On
Focal length	:	26 mm	Camera height	:	47.6 cm
Feature	:	HDR	Zoom	:	3.0 x

Calibration using the Spydercheckr48 color palette is performed on photos generated from the camera to reproduce colors better than in the original. Thus, it can better analyze chlorophyll or nitrogen content than without calibration. Calibration was done with Adobe Lightroom Classic version 11.4.1 (Adobe, San Jose, CA, USA) and Spydercheckr version 1.6 (Data color, Lawrenceville, NJ, USA). Figure 2 shows the difference in image color before and after calibration using Spydercheckr 48. Uncalibrated and calibrated images were then RGB-valued using ImageJ, an open-source image processing software (Schneider et al., 2012), and then converted to L*, a*, and b* values. The CIELAB color space (represented by L*, a*, and b* values) is considered superior for analyzing the color range from yellow to green in leaf color due to its enhanced perceptual uniformity compared to other color spaces, such as RGB or CMYK. The utilization of the CIELAB color space for the analysis of the yellow-to-green spectrum in leaf coloration facilitates the comprehension of the impact of chlorophyll content on the visual appearance of leaves. Correlation analysis and figures were generated using Origin Pro 2021 version 9.8.0.200 (OriginLab Corporation, Northampton, MA, USA).

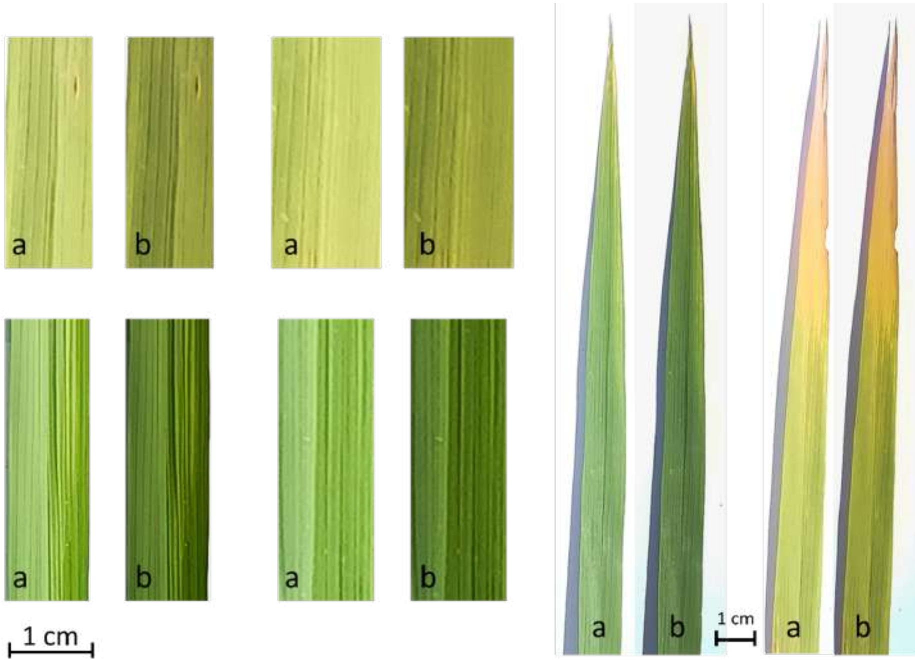

Figure 2. Images of leaf color digital image changes (a) before calibration and (b) after calibration using Spydercheckr 48

The acquired model was also employed to transform the non-calibrated camera images into SPAD values, nitrogen content, and chlorophyll content (a, b, and total chlorophyll). The values derived from the model were subsequently compared with the empirical values obtained from SPAD measurements and laboratory analysis, encompassing nitrogen, chlorophyll a, chlorophyll b, and total chlorophyll content. The accuracy computation involved the utilization of the discrepancy between the modeled and actual data.

RESULTS AND DISCUSSION

Relationship of SPAD and Nitrogen Content with Leaf Chlorophyll Content

The SPAD meter is a tool that enables rapid and non-invasive quantification of chlorophyll concentrations in plant leaves. This measurement provides a direct assessment of photosynthetic efficacy and the overall health of the plant. The correlation between chlorophyll and SPAD meters originates in the capacity of SPAD meters to offer a quantitative assessment of chlorophyll levels in plant leaves. SPAD meters provide the ability to assess plant health and productivity by quantifying leaf greenness. The utilization of this linkage has the potential to facilitate the monitoring of plant conditions, identification of nutrient deficiencies or plant stress, and provision of crucial information for the optimization of plant growth. There was a significant relationship between the SPAD value and chlorophyll content, encompassing chlorophyll a, b, and total. As the SPAD value increased, there was a corresponding increase in the chlorophyll content. The regression coefficients for chlorophyll a, b, and total were 0.6733, 0.5130, and 0.6422, respectively (Figure 3). The primary pigment responsible for absorbing sunlight during the process of photosynthesis in plants is chlorophyll. Consequently, greater SPAD values are indicative of more efficient rates of photosynthesis.

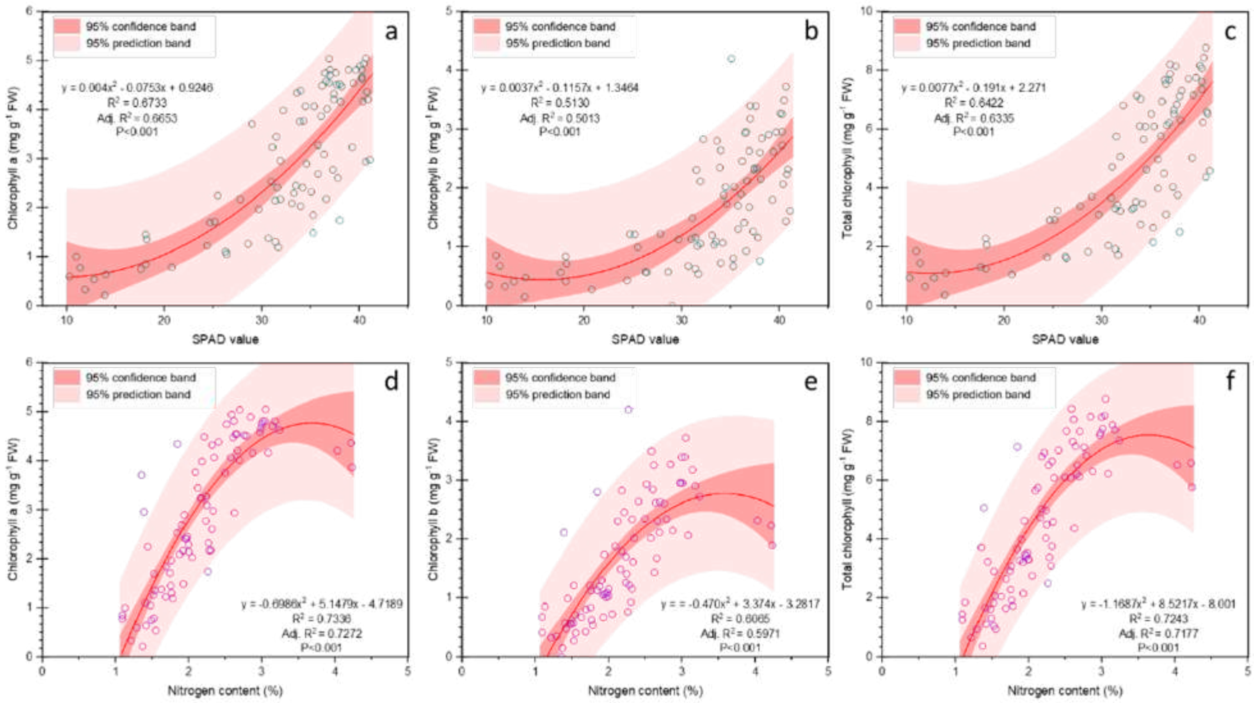

Figure 3. Relationship between SPAD and (a) chlorophyll a, (b) chlorophyll b, and (c) total chlorophyll, and between nitrogen content and (d) chlorophyll a, (e) chlorophyll b, and (f) total chlorophyll

Relationship of CIELAB Color Space with SPAD Value

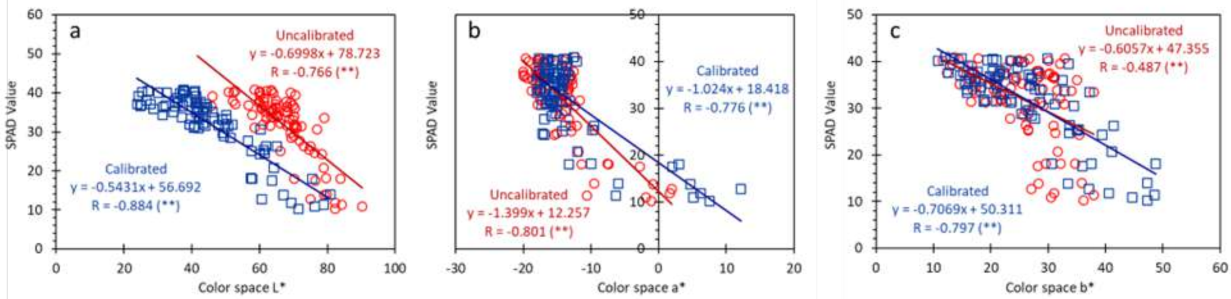

Figure 4. Comparison of correlation between SPAD and CIELAB color space values: (a) L*, (b) a*, and (c) b* in digital images, with and without calibration

The L*, a*, and b* color spaces had notable correlation coefficients with the SPAD value (Figure 4). The calibrated image exhibited a stronger correlation with the L* color space. The calibrated L* values exhibited a broader range than the uncalibrated model. In the context of the a* color space, the uncalibrated image had a slight advantage compared to the calibrated image despite both images demonstrating strong correlation values. The b* color space exhibited a significant rise, with the correlation value rising from -0.487 in the uncalibrated image to -0.797 after calibration. The color of the digital image demonstrated a strong correlation with the SPAD value, which became more pronounced when the digital image was calibrated. The correlation values between SPAD and L*, a*, and b* are high, exceeding those between SPAD and RGB on lettuce leaves, which were only 0.794, 0.346, and 0.387, respectively (Ibrahim et al., 2021).

Relationship of CIELAB Color Space with Nitrogen Content

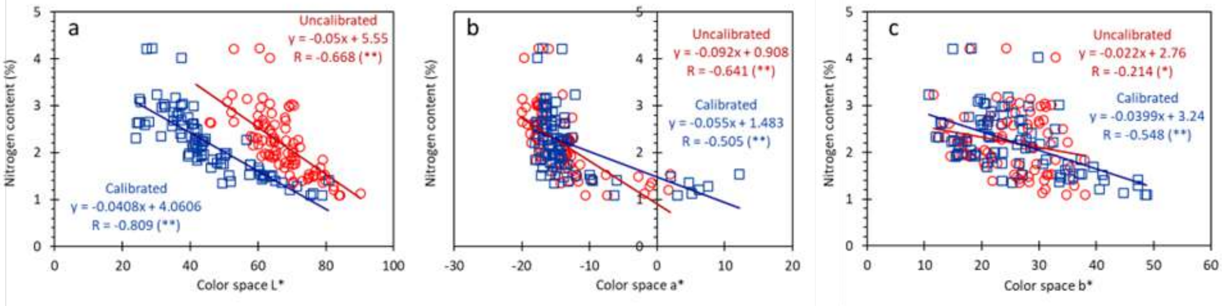

Figure 5. Comparison of correlation between nitrogen content and CIELAB color space values: (a) L*, (b) a*, and (c) b* in digital images, with and without calibration

Similarly, nitrogen content showed a significant correlation in all color spaces (Figure 5). The increase in correlation was found in the L* color space after calibration, from –0.668 to –0.809. When operating within the a* color space, the distinction between the calibrated and uncalibrated photographs was negligible. The b* color space demonstrated the most notable and prominent improvement. The uncalibrated digital image exhibited a correlation coefficient of -0.214, whereas the calibrated digital image demonstrated a correlation coefficient that was twice as large, measuring -0.548. The results indicated that the calibration process significantly improved the accuracy of the values in the digital image. The process of image calibration, when executed with accurate and well-defined color settings, is expected to produce an image that is identical to the original color of the sample. This is intended to enhance the accuracy of color information, hence facilitating the detection of nitrogen content.

Relationship of CIELAB Color Space with Chlorophyll a

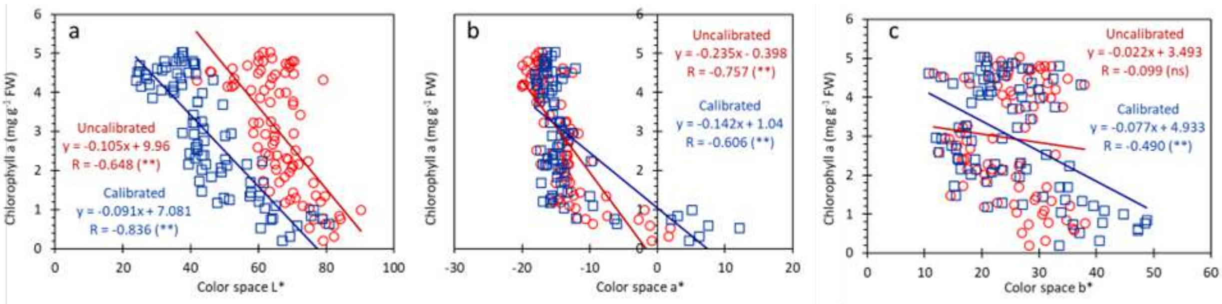

Figure 6. Comparison of correlation between chlorophyll a and CIELAB color space values: (a) L*, (b) a*, and (c) b* in digital images, with and without calibration

Chlorophyll a, being the primary pigment involved in the process of photosynthesis, plays an essential role in the absorption of solar energy. It possesses a distinctive ability to capture light throughout the red and blue wavelength ranges, typically spanning from approximately 430 to 662 nm (Bartolome et al., 2020; Zepka et al., 2019). The calibrated image exhibited a modest rise in the L* color space (R=-0.836, P<0.01) in contrast to the uncalibrated image (R=-0.648, P<0.01), despite both images displaying a strong correlation (Figure 6). This effect was also observed in the a* color space. In contrast, the b* color space exhibited a substantial enhancement in the calibrated image captured by a smartphone. Before the calibration, there was no substantial relationship between b* color space and chlorophyll a, as indicated by no significant correlation (R=-0.099, P>0.05). Nevertheless, the process of calibration utilizing Spydercheckr 48 demonstrated a strong correlation between b* and the camera that performed the calibration. The b* color space is a metric used to quantify the location of colors along the blue-to-yellow axis. Within the framework of b*, the horizontal axis of the Lab color space represents the blue-to-yellow axis, progressing from left to right. Positive values of b* are indicative of a greater yellow coloration, whilst negative values of b* are indicative of a greater blue coloration.

Relationship of CIELAB Color Space with Chlorophyll b

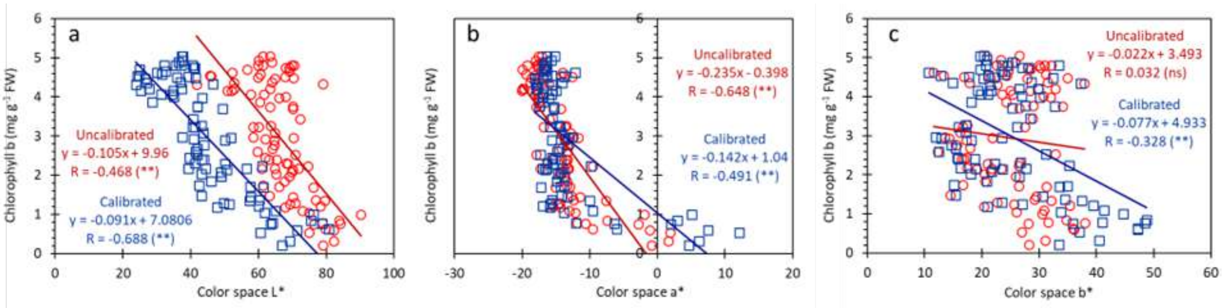

Figure 7. Comparison of correlation between chlorophyll b and CIELAB color space values: (a) L*, (b) a*, and (c) b* in digital images, with and without calibration

Chlorophyll b is a photosynthetic pigment that serves the purpose of absorbing light energy within the wavelength range of 453-642 nm, which is beyond the capacity of chlorophyll a. Chlorophyll b expands the range of light absorption in plants by effectively capturing light at distinct blue and red wavelengths. This broadening of the light spectrum enhances photosynthetic efficiency and confers an adaptive advantage in response to variations in external light conditions. Furthermore, the involvement of chlorophyll b in the energy transfer to chlorophyll a during the light-dependent phase of photosynthesis is important (Bartolome et al., 2020; Zepka et al., 2019). Similar to the correlation observed between the CIELAB color space and chlorophyll a, the association between the CIELAB color space and chlorophyll b exhibited a noticeable increase in the b* color space. Prior to calibration, the data exhibited an insignificant correlation, as shown by an R value of 0.032. However, following the calibration process, a significant correlation was observed, with an R value of -0.328 (Figure 7).

CIELAB Color Space Relationship with Total Chlorophyll

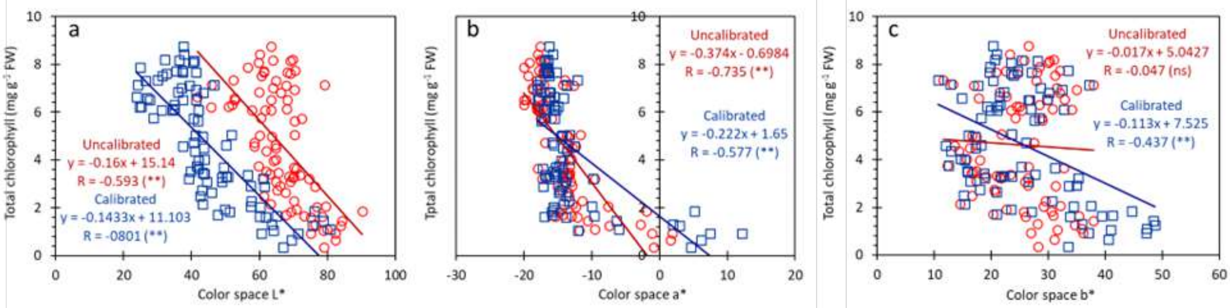

Figure 8. Comparison of correlation between total chlorophyll and CIELAB color space values: (a) L*, (b) a*, and (c) b* in digital images, with and without calibration

The aforementioned trend continued in relation to the aggregate measurement of chlorophyll, which includes both chlorophyll a and chlorophyll b. The correlation value in the L* color space exhibited a significant increase from R=-0.593 (P<0.01) to R=-0.801 (P<0.01) during the calibration process (Figure 8). The a* color space exhibited a minor decline, with an uncalibrated value of -0.735 (P<0.01), then decreased to -0.577 (P<0.01) when the image was performed calibration. In the same way, the b* color space demonstrated significant effects of digital image calibration, with a correlation coefficient of R=-0.047 (P>0.05) observed before calibration, which increased to -0.437 (P<0.01) after calibration.

Estimated SPAD, Nitrogen, and Chlorophyll Content using Smartphone Digital Image

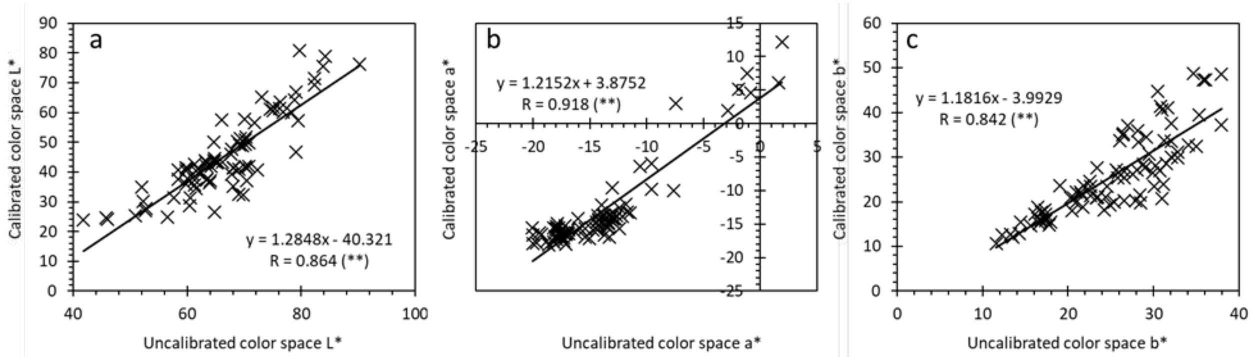

Figure 9. Correlation between calibrated and uncalibrated color space values: (a) L*, (b) a*, and (c) b*

The utilization of calibrated images indicating increased correlation across various variables (SPAD, nitrogen, chlorophyll a, chlorophyll b, and total chlorophyll) offers the possibility of facilitating the analysis of variable values. The process of calibrating an image is often complex due to the necessity of software assistance, which can be time-consuming and impractical for farmers. Hence, it is imperative to establish a correlation between the uncalibrated image and the analysis of crop variables. This can be achieved by correlating CIELAB values of the image data before and after calibration, resulting in the derivation of an equation. The L*, a*, and b* values showed significant correlation with coefficients of 0.864, 0.918, and 0.842, respectively (Figure 9). Color calibration is important because it is related to lighting conditions, where different light conditions allow the image color to be different, which in turn results in different accuracy (Astika & Khayati, 2019; Sunoj et al., 2018).

The trial results from 11 digital image samples showed that the accuracy of SPAD with the L*, a*, and b* color spaces was more than 85% (Table 2). This indicated that the CIELAB color space has the potential to assist observers in analyzing through smartphone cameras. Employing a color space alternative to RGB enables the attainment of an enhanced color range due to its expansive gamut of colors, perhaps leading to improved outcomes (Shrivastava & Pradhan, 2021; Sunoj et al., 2018). The CIELAB color space demonstrated an adequate degree of accuracy, averaging around 80% in most cases. However, it is worth noting that the b* color space exhibited a slightly lower accuracy rate of 79%. The use of CIELAB color space proves beneficial in the examination of nitrogen levels

Table 2. Accuracy analysis of color from trial samples of smartphone digital images

L* color space					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	92.3	84.7	74.6	73.5	75.7
Std. Dev.	7.0	9.1	14.0	16.0	14.8
RMSE	3.55	0.44	1.07	0.75	1.80
Min	76.3	68.9	48.5	51.0	53.4
Max	98.8	96.4	96.1	91.9	97.4
a* color space					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	87.9	81.9	70.6	69.9	70.8
Std. Dev.	8.9	13.4	23.0	18.8	18.2
RMSE	4.20	0.46	0.98	0.66	1.60
Min	66.5	52.1	19.0	26.2	34.5
Max	96.72	99.65	94.52	98.98	92.76
b* color space					
	SPAD	N content	Chlorophyll a	Chlorophyll b	Total chlorophyll
Average	87.9	79.0	65.3	66.4	66.1
Std. Dev.	8.9	10.0	18.5	12.7	13.5
RMSE	4.82	0.54	1.30	0.84	2.11
Min	74.4	62.5	30.5	54.1	44.1
Max	98.5	98.3	91.7	94.6	89.7

in rice leaves, particularly subsequent to the calibration of the image with a color standard. In contrast, the accuracy values of chlorophyll a and chlorophyll b were rather low, ranging from 62.3% to 74.6% in a* color space and from 66.4% to 73.5% in b* color space. The exacerbation of this issue was evident in the lowest accuracy values recorded in a* and b* color spaces, which demonstrated only 19.0% and 26.2%, respectively. Hence, it is imperative to implement enhancements in order to achieve a minimum accuracy threshold of 85%. Both chlorophyll a and b have an impact on the overall chlorophyll measurement. The accuracy of total chlorophyll is relatively low because of the low accuracy values of chlorophyll a and b. The L* color space exhibited an accuracy value of 75.7%, whilst the a* and b* color spaces demonstrated accuracies of 70.8% and 66.1%, respectively.

CONCLUSION

There was a significant correlation between plant leaf greenness variables (SPAD, N content, chlorophyll a, b, and total chlorophyll) and images obtained from smartphones based on the CIELAB color space. The correlation value increased when calibration was done first using a color palette. With the high correlation value, the use of smartphone camera images has great potential in estimating these plant greenness variables. The accuracy of SPAD and nitrogen content values was high, ranging from 79.0% to 92.3%, depending on the L*a*b color space used. Meanwhile, it is observed that the accuracy values for chlorophyll a and b remain relatively low, ranging from 65.3% to 74.6%. Similarly, the accuracy of the total chlorophyll content was reported to be between 66.1% and 75.7%.

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AUTHORS CONTRIBUTIONS

Conceptualization was led by VK, KF, and AIU; methodology was developed by VK and KF; validation was carried out by KF and AIU; analysis was performed by VK, KF, LK, and F; the investigation was conducted by VK, KF, LK, and F; data curation was managed by LK and F; the original draft was prepared by VK and KF; review and editing were undertaken by VK and AIU. All authors provided valuable feedback and made significant contributions to the research development, analysis, and manuscript preparation.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Floral Morphological Variation in Black Pepper (*Piper nigrum* L.) Varieties and Hybrid Lines

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ABSTRACT

The inter-variety crossing is a promising approach to increase black pepper production. However, successful hybridization hinges on floral characteristics. This study investigated the floral morphology of diverse pepper varieties and their hybrid offspring. Eighteen genotypes, including parent varieties and F1 hybrids, were cultivated in pots under greenhouse conditions at the Indonesian Spice and Medicinal Crops Research Institute, Bogor. Flower and fruit traits were observed. Results indicated variability in spike morphology. While most ripe spikes were yellowish-green, exceptions included LH 20-1 and LH 22-1. Ciinten and LH 4-5 displayed superior spike length and fruit set, contrasting with the shorter, less productive spikes of 20-1, 22-1, and 455-N2-97. Stigma receptivity and another dehiscence coincided in Ciinten, N2BK, LH 6-2, and LH 4-5, simplifying the hybridization process. Conversely, earlier stigma receptivity in Petaling 1, Petaling 2, Natar 1, Natar 2, LH 44-9, LH 20-1, and LH 22-1 facilitated castration procedures. These findings offer valuable insights for optimizing pepper breeding programs.

Keywords: Castration; Flower characteristics; Inter-varietal hybridization; Spices; Stigma receptivity

INTRODUCTION

Black pepper (*Piper nigrum* L.) is the world's most traded spice. Indonesia is one of the world's largest spice producers, with major commodities being black pepper, nutmeg, clove, cardamom, and cinnamon. Black pepper is native to the Western Ghats of India (Kumar et al., 2021), but nowadays, it has already been widely cultivated in several countries, including Indonesia. In Indonesia, black pepper has been cultivated in several provinces, such as Bangka, Lampung, South Sulawesi, East Kalimantan, South East Sulawesi, and West Java. Two product types of pepper are famous in the market, namely black and white pepper. White pepper is usually processed from varieties with big berries, such as Petaling and Ciinten. Meanwhile, black pepper is processed from varieties with smaller berries, such as Natar-1 and Natar-2. Currently, ten high-yielding varieties have been released, namely (1) Petaling 1, (2) Petaling 2, (3) Natar 1, (4) Natar 2, (5) Lampung Daun Kecil, (6) Chunuk, (7) Bengkayang, (8) Ciinten, (9) Malonan, and (10) Nyelungkup. Black pepper has several biological roles, including antioxidant, anti-inflammatory, anticancer, anti-obesity, antidepressant, antidiabetic, antimicrobial, gastroprotective, and insecticidal activities (Ashokkumar et al., 2021).

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The main constraints in black pepper cultivation are low productivity compared to the other producing countries, stem borer (Laba, 2018; Manohara, 2013), and foot rot disease (Manohara, 2013; Prasmatiwi & Evizal, 2020). Black pepper productivity in other pepper-producing countries could reach 3098 kg/ha in Brazil and 4650 kg/ha in Malaysia (FAOSTAT, 2022). Low productivity and the absence of pest, disease, and virus-resistant varieties may be due to the inadequate genetic variability within the cultivated varieties in the *Piper nigrum* species populations (Manohara, 2013).

Therefore, it is necessary to broaden genetic variability through intercrossing between varieties or species. However, successful hybridization may be obtained from understanding flower biology, especially the time of anthers dehiscence and stigma receptivity. Therefore, before inter-variety hybridization is carried out, studying biology and the morphological characteristics of pepper flowers is essential.

Apart from that, the choice of parents to be crossed is essential. The parents selected must have superior characteristics according to the breeding objectives to be achieved so that the hybrids produced would gain a good combination of characteristics from both parents.

Yield per vine correlated with the number of spikes per plant, the spike length, the number of fruits (berries) per spike, and the size. The more extended spike with many berries tends to produce a high yield. According to Bermawie et al. (2019), the ideotype of black pepper plants is those with good plant appearance with more branches, more prolonged spikes (≥ 12 cm) with a high number of berries (≥70) per spike, and big berry size. Moreover, berry size is related to the pepper products produced. There are two major pepper products in the international market, namely black and white pepper. White pepper is usually processed from varieties with big berries, while black pepper is processed from smaller ones.

The inflorescence characteristics are an important characteristic of the pepper variety. Pepper flower inflorescence is formed on fruit branches. Its position is the opposite of the petiole and is called a spike, but not every leaf produces a spike. The flower consists of female, male, and bisexual flowers (Wulandari et al., 2021). The stamen flanks the stigma on both the left/right sides. A larger size and whitish color characterize stigma receptivity. Mature stigma starts from the base of the spike. The ripe stamen looks white, protruding beside the stigma.

Flower characteristics in black pepper are often correlated with yield per vine, such as spike length, number of flowers per spike, and berry size. Several inter-variety crosses have been obtained. It is necessary to study the flower characteristics, that may be related to their potential yield and the most suitable recommended pepper products. This study aimed to observe the morphological characteristics of flowers for their potential success in hybridization and their further correlation with the potential yield per vine. This research can provide fundamental information to determine effective and efficient pepper breeding strategies, especially the crossing stage.

MATERIALS AND METHODS

The study was conducted in the greenhouse of the Indonesian Spice and Medicinal Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Bogor, West Java, Indonesia, from January 2020 to December 2021.

Preparation of Planting Materials

Eighteen genotypes were used in this study, consisting of eight high-yielding varieties (Ciinten, Belantung, Lampung Daun Lebar, Bengkayang, Petaling 1, Petaling 2, Natar 1 dan Natar 2) and ten hybrid lines that have been screened foot rot diseases (LH 4-5, LH 4-5-5, LH 6-2, LH 20-1, LH 22-1, LH 37-16, LH 44-9, N2BK, LH N2BK x LDL-1, and LH 4-5-5x N2-97).

The plant material used was bushy pepper. The plants were prepared from fruit branches taken from each variety/genotype of mature trees. The branches were cut and then planted in a big pot 40 cm in height x 30 cm in diameter, containing soil and cow dung mature in a 1:1 ratio. Each genotype consisted of 5 pots. Plant care followed the procedures for bushy peppers. The plants began bearing flowers at 3 years old, and observation began.

Observation of Morphological Characteristics

Fifty mature spikes were randomly selected per each genotype/variety and used for observation.

Observation of Qualitative Morphological Characters

The selected spikes were observed for qualitative characteristics, such as spike color, the dense flower arrangement in the spike, time of stigma receptivity, and stamen dehiscent for fertilization.

Spike Color

The RHS color chart was used to observe the color by comparing the color of the spike with the color on the RHS color chart.

The Dense Flower Arrangement

The density of flower arrangements in the spike was observed visually with the help of a head magnifier. The arrangement of the stigmas in a spike was categorized as dense or moderate (relatively sparse).

Time of Stigma Receptive and Stamen Dehiscent

The timing of stigma receptivity and another dehiscence was observed visually using a head magnifier. The stigma elongating indicates stigma receptivity, fully open flowers, and a slightly white color. The theca flanking and the anthers dehiscing indicate stamen ripeness. The synchronization of stigma receptivity and anther dehiscence was observed in two scenarios: (1) on the same day or (2) with anther dehiscence occurring 1-2 days later.

Observation of Quantitative Morphological Characters

The quantitative morphological characteristics observed were peduncle length, spike length, spike diameter, number of flowers per spike, and number of berries per spike. The spike was observed when the stigma was in a mature stage.

Data Analysis

Qualitative morphological characteristics data were analyzed using descriptive statistics, and data of quantitative characteristics were analyzed using variance and correlation analysis.

RESULTS AND DISCUSSION

The spike characteristics of pepper varieties and hybrid lines are presented in Table 1. The spike characters of varieties and hybrid lines varied. Spike color mainly was yellowish green, but three accessions (LH 20-1, LH 22-1, LH N2BK) had a light-yellow color, and after pollination, the color became brownish yellow (YGG 153 D). The spike color of the black pepper of Malaysian cultivar was green, whitish green, or light yellow (Chen & Tawan, 2020a), with the color of inflorescence included in the Green group N144, Green group 144, and Green group 145 (Chen & Tawan, 2020b). Wulandari et al. (2021) reviewed that the color of the inflorescence of black pepper was light green-yellow. Pooja (2019) reported that the spike color of Panniyur 1 was light green and dark green. Variations in the color of the spike may be attributed to genetic differences.

Table 1. Qualitative characteristics of spike in several black pepper varieties and hybrid lines

No	Variety/lines	Young spike color	Mature spike color	Time of stigma receptivity vs anther dehiscence	Stigma size	Flower arrangement on the spike
1	Ciinten	YGG 145 A-B	YGG N144 D	Same time	medium	dense
2	Belantung	YGG 145 A-B	YGG N144 D	early	medium	moderate
3	Lampung Daun Lebar	YGG 145 B-C	YGG N144 D	Same time	medium	moderate
4	Bengkayang	YGG 145 A-B	YGG N144 D	early	medium	moderate
5	Petaling1	YGG 145 A-B	YGG N144 D	early	medium	moderate
6	Petaling 2	YGG 145 B-C	YGG N144 D	early	medium	moderate
7	Natar 2	YGG 145 A-B	YGG N144 D	early	medium	moderate
8	Natar 1	YGG 145 A-B	YGG N144 D	early	medium	moderate
9	LH 4-5	YGG 145 A-B	YGG N144 D	same	medium	dense
10	LH 4-5-5	YGG 145 A-B	YGG N144 D	same	medium	moderate
11	LH 6-2	YGG 145 A-B	YGG N144 D	same	medium	moderate
12	LH 20-1	YGG 145 A-B	YGG 153 D	early	big	dense
13	LH 22-1	YGG 145 B-C	YGG 153 D	early	big	dense
14	LH 37-16	YGG 145 A	YGG N144 D	early	small	dense
15	LH 44-9	YGG 145 A-B	YGG N144 D	early	Big and thick	moderate
16	LH N2BK	YGG 145 A-B	YGG 145 B-C	early	medium	moderate
17	LH N2BK x LDL-1	YGG 145 A-B	YGG N144 D	early	medium	moderate
18	LH 455-N2-97	YGG 145 A-B	YGG N144 D	early	big	dense

Note: YGG = yellow-green group

Anther dehiscence in the studied pepper varieties and F1 lines typically occurred at 10-11 AM on sunny days, with delays under cloudy conditions. However, this timing varies geographically. In Vietnam, Vinh Linh and Phu Quoc varieties dehisce earlier at 7-8 AM (Quyen et al., 2019). Indian Panniyur-1 exhibits a distinct pattern, with anthesis starting in the afternoon and anther dehiscence peaking between 2-3 PM (Pooja, 2019). Generally, black pepper anther dehiscence falls around 11-12 AM, with pollen viability lasting up to ten hours (Chen et al., 2018). Precise knowledge of anther dehiscence timing in both cultivated and wild peppers is crucial for successful interspecific crosses. It's important to note that both genetic factors and environmental conditions influence these timings.

The time of stigma receptivity versus anther dehiscence is one of the essential characteristics in determining the success of hybridization. Stigma receptivity and anther dehiscence are categorized as early, simultaneous, or late. In most varieties, stigma receptivity occurred one day earlier than anther dehiscence (Table 1), indicating geitonogamy (Pooja, 2019). Varieties with stigma receptivity occurring earlier than anther dehiscence are beneficial for artificial crossing, as this timing can minimize mechanical damage to spikes and stigmas due to accidental contact during castration. The varieties with earlier stigma receptivity than anther dehiscence are better used as the female parent.

On the other hand, in hybridization involving varieties where anther dehiscence occurs earlier than stigma receptivity, it is preferable to use these varieties as the male parent. If the varieties are used as the female parent, the risk of failure is higher due to unavoidable castration. The stigma becomes damaged/dry from being accidentally touched during castration and is no longer receptive. Varieties and hybrids with anther dehiscence occurring at the same time as stigma receptivity include Ciinten, Lampung Daun Lebar, LH 4-5, LH 4-5-5, and LH 6-2. The Ciinten variety, which produces abundant pollen, is best used as a male parent. However, varieties with simultaneous stigma receptivity and anther dehiscence have a higher risk of self-pollination contamination and a reduced success rate for crosses due to accidental touch or injury during castration activities.

Stigma size also influences the success rate of hybridization. Varieties with large stigma sizes can increase the chances of sticking pollen during hybridization. Hybridization is more manageable in a hybrid line with an enormous stigma size, and the potential for success is high. However, in all crosses involving LH 44-9 with a big size stigma, as a female parent, it is easy to cross, but the success rate is low. Additionally, LH 44-9 has a moderate bloom density therefore, there are no issues with the castration procedure. The low success rate in crosses involving LH 44-9 may be attributed to other factors, such as the sterility of female flowers or other genetic factors that need further study. LH 44-9 is a hybrid of a cross between LDK and P2.

Table 2. Quantitative morphological characteristics of spike in several black pepper varieties and hybrid lines

No	Variety	Peduncle length (cm)	Spike length (cm)	Spike diameter (mm)	Numbers of flower	Numbers of berries
1	Ciinten	1.11abc	9.24a	3.24bc	107.25a	87.00a
2	Belantung	0.84def	6.75bcde	3.22bc	70.49def	34.67cdef
3	Lampung Daun Lebar	0.90cdef	6.20def	3.05cd	61.94ef	26.17efgh
4	Bengkayang	0.98bcde	5.98def	3.01cd	60.80ef	31.85defg
5	Petaling1	0.92cdef	6.73bcde	2.70d	75.02cd	35.94cde
6	Petaling 2	0.96bcde	6.3cdef	3.07cd	61.18ef	35.22cde
7	Natar 2	0.88cdef	6.82bcd	3.05cd	84.94bc	39.30cd
8	Natar 1	0.91cdef	5.32f	3.09cd	58.90f	30.26defg
9	LH 4-5	1.06abcd	9.64a	3.27bc	107.24a	63.84b
10	LH 4-5-5	1.06abcd	7.60b	3.23bc	92.08b	42.00c
11	LH 6-2	1.19ab	7.33bc	3.55ab	75.32cd	30.53defg
12	LH 20-1	0.80ef	3.26g	2.94cd	33.44g	16.79hij
13	LH 22-1	0.72f	3.40g	3.07cd	37.68g	15.18ij
14	LH 37-16	1.09abc	6.36cde	2.95cd	66.13def	25.11fgh
15	LH 44-9	1.23a	7.29bc	3.75a	72.10cde	7.78jk
16	LH N2BK	0.98bcde	5.73ef	2.74d	60.76ef	23.90ghi
17	LH N2BKxLDL-1	1.08abc	3.66g	2.71d	28.16g	-
18	LH 4-5-5 x N2-97	1.02abcde	3.26g	3.07cd	35.43g	3.88k

Note: Means followed by different letters are significantly different (Tukey HSD Test, α = 0.05)

LH 37-16 has a smaller stigma and high flower density in the spike. The smaller size of the stigma and the high density of the flower in spike are unfavorable, mainly if it is used as a female. A high density of flowers causes difficulty during castration. The risk of pistil damage is high from accidentally touching it during the castration process.

Natar 1, Natar 2, Petaling 1, and Petaling 2 have ideal flower characteristics for hybridization, with earlier stigma receptivity than anther dehiscence and moderate flower density, so a high success rate of crosses was obtained.

Spike length and number of flowers per spike vary among varieties and lines (Table 2). These characteristics are related to high yield per vine. The spike length from these studies ranges from 3.26 – 9.64 cm, with the numbers of flowers and berries of 28-107 and 4-87, respectively. Most varieties and lines have a moderate length of the spike. Varieties that have long spikes are Ciinten, followed by hybrid lines of LH 4-5 and LH 44-9. The hybrid lines with short spike lengths include LH 20-1, LH 22-1, and LH 455-N2-97. In the form of climbing pepper, the Ciinten variety has a spike length of 11 cm and a high number of berries, higher than the other superior varieties (Bermawie et al., 2019; Meilawati et al., 2020). There is no difference between Ciinten grown as a shrub and a climbing plant. Spike length and number of fruits (berries) per spike can be used to distinguish varieties (Chen & Tawan, 2020b). Spike character is directly related to attributes of various black pepper branch types (Bhasi et al., 2017).

Generally, a longer spike tends to have more flowers and berries. However, in LH 44-9, despite having a long spike and many flowers, the fruit set is low. The number of fruits per spike is less than 10. It may be caused by anther dehiscence of LH 44-9 is later, almost two days later than stigma receptivity. The first stigma appears to elongate in two days and continues to emerge wide 2-4 days later (Chen et al., 2018). The viability of pollens is high, which is up to ten hours after anther dehiscence. Stigma is the most receptive when it is in a fully emerged stadium. In LH 44-9, the duration for self-pollination is shorter because the time of stigma receptivity differs from anther dehiscence, with stigma receptivity occurring much earlier (by two days). The low fruit set may also be due to stigma damage from contact with nearby leaves or passing insects.

Spike length and the number of berries per spike are critical determinants of black pepper yield (Bermawie et al., 2019; Shango et al., 2021), in which spike length is categorized into short (<10 cm), medium (10-15 cm), and long (>15 cm) groups. A positive correlation has also been established between berry count per spike and pepper yield (Bermawie et al., 2019). The Ciinten variety exhibited a spike length of 9.24 cm with 87 berries, while other varieties and F1 hybrids in this study displayed shorter spikes. This indicates a narrow genetic base for spike characteristics among the parental lines. Incorporating parents with longer spikes into breeding programs to increase black pepper yield is essential for improving spike length.

Quality parameters such as essential oil, piperine, and oleoresin are important for pepper. Ciinten is a high-yielding variety with a berry yield of 4.30 kg per vine, piperine content of 4.73%, essential oil content of 2.79%, and oleoresin content of 15.98% (Bermawie et al., 2019). In Tanzania, the black pepper length of spikes is 5.6-12.4 cm, with the number of flowers varying from 55.4-93.5, and the percentage of fruit set of 46.7-86 %, depending on the varieties (Shango et al., 2021). In India, Panniyur-1 has flowers ranging from 48 to 98, depending on the spike length, with a percentage of fruit set at 92.90% (Pooja, 2019). Meanwhile, the cultivar Kurimale in Karnataka has a spike length of 22.99 cm and produces many berries (103.7) (Pannaga et al., 2021). Studies on the breeding system in *Piper nigrum* revealed that high fruit sets resulted from geitonogamy, followed by autogamy, open pollination, and xenogamy (Pooja et al., 2022).

There are eight genotypes with long peduncles, namely LH 44-9, LH 6-2, Ciinten, LH 37-16, LH N2BKxLDL-1, LH 455-N2-97, LH 4-5, and LH 4-5-5. LH 44-9 has the longest peduncle and is significantly different from Belantung, Lampung Daun Lebar, Bengkayang, Petaling 1, Petaling 2, Natar 1, Natar 2, LH 20-1, LH 22-1, and N2BK based on Tukey test at 95% (Table 2). Ciinten is a superior pepper variety with the most extended spike length. LH 4-5 and Ciinten have the most extended spike and significant numbers of stigma. This study also shows that the Ciinten produces the highest number of berries. These varieties and lines are significantly different from each other. Although LH 4-5 has fewer fruits than Ciinten, it still has many fruits and differs significantly from other varieties and lines. LH 44-9 has the largest spike diameter, significantly differing from other varieties and lines. Meanwhile, LH 22-1 has the shortest peduncle, the shortest spike, and fewer flowers.

Table 3. Correlation between spike quantitative characteristics

Trait	Peduncle length (cm)	Spike length (cm)	Number of stigma	Spike diameter (mm)	Number of berries per spike
Peduncle length	1				
Spike length	0.19	1			
Number of stigma	0.15	0.93	1		
Spike diameter	0.17	0.17	0.08	1	
Number of berries per spike	0.08	0.60	0.64	-0.04	1

Based on this study, the quantitative characteristics are positively correlated except between fruit numbers per spike and spike diameter (Table 3). The correlation between the numbers of stigma and spike length is very strong, about 0.93. It means that the longer the spike, the more stigma. Spike length, numbers of stigma, and fruit numbers per spike are very strongly correlated. These characteristics are also the main yield component characteristics in pepper.

The spike length is positively correlated with the number of berries/spikes of black pepper. It follows previous research on black pepper in Ethiopia (Bekele et al., 2017). Spike length also has a significant and positive correlation with the starch content of leaves (Zu et al., 2018). A positive correlation between spike length and yield was also observed (Shango et al., 2021; Shivakumar & Saji, 2019). This characteristic has high heritability (Preethy et al., 2018). The number of berries per spike or percentage of fruit set for a yield of black pepper was also reported by Prayoga et al. (2020) and Oanh et al. (2021). However, the number of fruits per spike is negatively correlated with spike diameter, although the correlation is weak. This condition is a challenge for breeders in increasing pepper production because spike diameter is also one of the yield component characteristics in pepper.

CONCLUSION

The morphological characteristic of spikes varies among varieties and lines. The color of ripe panicles is generally yellowish green, except for LH 20-1 and LH 22-1. Ciinten and LH 4-5 varieties have the longest spike and the highest fruit sets. LH 20-1, LH 22-1, and L 4-5-5 x N2-97 have short spikes and low fruit sets. Stigma receptivity and anther dehiscence in spikes occur simultaneously in Ciinten, N2BK, LH 6-2, and LH 4-5. In contrast, in Petaling 1, Petaling 2, Natar 1, Natar 2, LH 44-9, LH 20-1, and LH 22-1, stigma receptivity precedes anther dehiscence. This earlier stigma receptivity facilitates easier castration. This finding will increase the effectiveness and success of pepper breeding programs.

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AUTHORS CONTRIBUTIONS

SW conducted research, observation, and data interpretation and wrote the original draft. NB conceptualized, supervised, observed, interpreted data, wrote, reviewed, and edited the original draft. NS conducted research and observation. MS conducted research, observation, data analysis, and data interpretation and wrote and edited the original draft.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abundance, Attack Intensity, and Distribution of *Spodoptera frugiperda* J.E. Smith in Kulon Progo, Yogyakarta

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ABSTRACT

Spodoptera frugiperda is an invasive corn pest from America and has entered Indonesia, including Kulon Progo. However, the abundance, attack intensity, and distribution of *S. frugiperda* have never been reported from Kulon Progo. This study aimed to analyze the abundance, attack intensity, and distribution of *S. frugiperda* larvae in Kulon Progo. This research was conducted from January to March 2023. Sampling locations were determined using the purposive sampling method. The egg packages and larvae of *S. frugiperda* found were counted to determine the abundance of *S. frugiperda*. The intensity of *S. frugiperda* infestation was calculated using the letter W scouting method. The results showed that 24 *S. frugiperda* egg packages were obtained in Kulon Progo, and the abundance of *S. frugiperda* was 0.01 individuals per plant. The attack intensity of *S. frugiperda* in Kulon Progo reached 4.97%, with the highest attack intensity in Nanggulan (8%) and the lowest in Wates (1.5%). The larvae of *S. frugiperda* are distributed in four sub-districts in Kulon Progo. This study concluded that the attack intensity of *S. frugiperda* in Kulon Progo was included in the mild category.

Keywords: Fall army worm; Kulon Progo; Larva; Pest; Scouting

INTRODUCTION

Fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is an invasive pest from America and has spread to various countries (Sharanabasappa et al., 2018; Pratama et al., 2020), including Indonesia (Nonci et al., 2019). *Spodoptera frugiperda* can attack various plants, including food crops (Simanjuntak et al., 2022), horticulture (Irawan et al., 2022), and plantations (Arsi et al., 2021). Corn is one of the food crops reported to be attacked by *S. frugiperda* (Maharani et al., 2019).

Spodoptera frugiperda attacks corn plants from the vegetative to the generative phase (O Awata et al., 2019), with the highest damage occurring in the vegetative phase (Trisyono et al., 2019). The larvae of this pest attack the growing point of corn plants, resulting in the failure of young leaf formation (Maharani et al., 2019). The leaves of corn plants infested by *S. frugiperda* are characterized by holes left by larvae (Novita et al., 2021) and larval feces (Arfan et al., 2020). *Spodoptera*

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frugiperda has been reported to cause corn yield losses of 8.3 - 20.6 million tons/year or US$ 2.5 - 6.2 billion per year in Africa and Europe (FAO & CABI, 2019). Yield losses in corn crops due to *S. frugiperda* infestation have been reported in various countries, such as Brazil (34 %), Zimbabwe (11.57 %) (Baudron et al., 2019), Kenya (>30 %) (De Groote et al., 2020), and India (33 %) (Balla et al., 2019). Megasari and Khoiri (2021) also reported Indonesia's highest corn yield loss due to *S. frugiperda* infestation reaching 40% in Tuban District, East Java.

Provinces in Indonesia that have reported *S. frugiperda* infestation on corn plants include Lampung (Trisyono et al., 2019), West Java (Maharani et al., 2019; Sartiami et al., 2020), Banten and West Sumatra (Sartiami et al., 2020), East Kalimantan (Subiono, 2020), and East Java (Megasari & Khoiri, 2021). In addition, Nurkomar et al. (2021) also reported the presence of *S. frugiperda* in Bantul and Sleman, Special Region of Yogyakarta, with larval densities ranging from 0 - 1 larva per plant. However, the population and attack intensity of *S. frugiperda* infestation have not been reported from Kulon Progo, Yogyakarta. The Department of Agriculture and Food Security of Yogyakarta has reported *S. frugiperda* infestation in several areas in Kulon Progo, including Temon, Wates, Panjatan, Pengasih, and Sentolo (DPKP DIY, 2019). Therefore, this research was conducted as an initial population database to prevent the *S. frugiperda* population explosion in Kulon Progo, Yogyakarta.

MATERIALS AND METHODS

Determination of Sampling Locations and *S. frugiperda* Sampling

Figure 1. Sampling location map in Kulon Progo

The research was conducted in Kulon Progo, Yogyakarta, from January to June 2023. The number of *S. frugiperda* eggs was determined at Ahmad Dahlan University's Ecology and Systematics Research Laboratory, part of the Biology Study Program. This study used a purposive sampling method to determine the sampling location. In each sub-district in Kulon Progo, two villages that meet the research criteria were selected. The villages chosen were the villages that had a corn field with at least 50 corn plants that showed symptoms of *S. fugiperda* attack. The coordinate points of the selected corn fields were recorded using GPS essential (Figure 1). Samples of *S. frugiperda* larvae were collected directly. The obtained samples were put into plastic to be counted and identified.

The Calculation of Attack Intensity of *S. frugiperda*

The scouting method of the letter W was used to calculate the attack intensity of *S. frugiperda*. Plants exhibiting signs of an attack by *S. frugiperda* were tallied and documented. The calculation of attack intensity was calculated using the formula according to Megasari & Khoiri (2021), namely:

$$P = \frac{n}{N} x\ 100\% \qquad (1)$$

P = attack intensity
n = number of plants that attacked
N = total of plants that counted in the sampling location

After calculating the attack intensity, the intensity value was grouped according to Pratama et al. (2020) (Table 1).

Table 1. Attack intensity category of *S. frugiperda* in corn plant (Pratama et al., 2020)

Skala	Percentage (%)	Criteria
0	0	No attack
1	0 < x < 25	Light
2	25 < x < 50	Medium
3	50 < x < 75	Heavy
4	x ≥ 75	Extremely heavy

Making a Distribution Map of *S. frugiperda*

The application that was used to create the attack intensity map was ArcGIS. The administrative map of district and sub-district boundaries in Kulon Progo Regency was downloaded, and then the administrative map was put into the software. The sub-district boundary layers were Right-clicked, the attribute table was entered, and fields were added to add data on the attack intensity of *S. frugiperda*. The properties option on the sub-district boundary layers was right-clicked, symbology was clicked, and quantities were chosen to set the appropriate value and class. Then, layers were right-clicked, and the table was checked to display the name of each sub-district. Digitization was organized by clicking on the insert to add the map title, cardinal directions, numerical and pictorial scale, legend, and netline. Last, the map was saved as .jpeg or .jpg by clicking on the export map file.

Data Analysis

This study employed both descriptive and inferential data analysis. The variations in *S. frugiperda* abundance in Kulon Progo were explained using descriptive analysis. Inferential analysis was used to calculate the percentage of *S. frugiperda* attack intensity on corn plants in Kulon Progo.

RESULTS AND DISCUSSION

Abundance of *S. frugiperda* on Corn Plant in Kulon Progo

Based on the results of this research, a total of 24 *S. frugiperda* egg packages were obtained in Kulon Progo. The number of eggs obtained was 2103 eggs. The total abundance of *S. frugiperda* was 0.01 individuals per plant, consisting of five 2nd instar larvae and seven 3rd instar larvae (Table 2).

Table 2. Abundance of *S. frugiperda* individuals attacking corn plants in Kulon Progo

Subdistrict	Number of eggs	Number of *S. frugiperda* larvae per plant
Sentolo	337.5	0
Galur	0	0
Pengasih	0	2.5
Wates	121	0
Kalibawang	0	1.5
Nanggulan	145.5	1
Girimulyo	0	0
Lendah	0	0.5
Panjatan	0	0
Temon	368	0
Kokap	79.5	0
Samigaluh	0	0.5

The results of the research found that the abundance of *S. frugiperda* larvae obtained was less than the number of egg packages. This is because the *S. frugiperda* individuals obtained were generally from 2nd and 3rd instar larvae, cannibals (Pebrianti et al., 2021). Due to this cannibal nature, only one to two 2nd or 3rd instar larvae were found on one corn plant (Bakry et al., 2023). According to the research of Prasetya et al. (2022) and Nonci et al. (2019), the cannibalism of *S. frugiperda* larvae causes only one to two 2nd and 3rd instar larvae to be found on one corn plant. Meanwhile, the sampling time in this study also affected the abundance of *S. frugiperda* larvae (Widhayasa & Suryadarma, 2021). This research was conducted during the rainy season, from December 2022 to March 2023. This seasonal condition is thought to affect the abundance of *S. frugiperda* larvae because they are washed to the ground (Caniço et al., 2020). In addition, rainwater entering the base of the corn plant can cause 3rd instar larvae to drown in their burrows (Maharani et al., 2019). At the time of sampling, many of the corn plants in Kulon Progo had entered the generative phase. Due to the generative phase, the number of *S. frugiperda* larvae decreased (Zeni et al., 2021). This is consistent with the study by Jaramillo et al. (2019), who discovered a tiny population of *S. frugiperda* in corn plants during their generative phase.

In contrast, the number of *S. frugiperda* egg packages found at the sampling location was larger than the abundance of individuals, which was 24 egg packages. According to Prasetya et al. (2022), females of *S. frugiperda* imago tend to lay eggs on corn plants that have entered the generative phase. In addition to the phase preference of corn plants, *S. frugiperda* females are attracted by compounds released by corn plants when entering the generative phase (Listyawati et al., 2021). These compounds are phenol compounds, one of which is vanillic acid, which is an attractive compound for *S. frugiperda* females to lay their eggs on corn plants (Anisa et al., 2023).

Table 3. Abiotic factors measured in each district in Kulon Progo

Subdistrict	Abiotic factors		
	Air temperature (°C)	Air humidity (%)	Light intensity (Lux)
Sentolo	35.71	51.83	79003.33
Galur	28.53	84.83	763516.67
Pengasih	33.66	58.50	73478.33
Wates	34.54	60.83	88240.00
Kalibawang	32.88	71.33	278266.67
Nanggulan	35.63	45.17	80965
Girimulyo	29.20	77	21583.33
Lendah	31.88	86.33	37933.33
Panjatan	32.44	85.17	36120.83
Temon	33.03	66.83	63715.00
Kokap	34.27	74.67	40393.33
Samigaluh	30.18	76.33	25498.33

Table 4. Correlation between the number of *S. frugiperda* eggs and abiotic factors

			Number of Eggs	Air Temperature	Air Humidity	Light Intensity
Spearman's rho	Number of Eggs	Correlation Coefficient	1.000	.537**	-.663**	.073
		Sig (2-tailed)	.	.008	.001	.740
		N	23	23	23	23
	Air Temperature	Correlation Coefficient	.537**	1.000	-.776**	.156
		Sig (2-tailed)	.008	.	.000	.477
		N	23	23	23	23
	Air Humidity	Correlation Coefficient	-.663**	-.776**	1.000	-.178
		Sig (2-tailed)	.001	.000	.	.415
		N	23	23	23	23
	Light Intensity	Correlation Coefficient	.073	.156	-.178	1.000
		Sig (2-tailed)	.740	.477	.415	.
		N	23	23	23	23

Note: ** Correlation is significant at the 0.01 level (2-tailed)

According to Table 2, the number of eggs and the number of S. frugiperda larvae obtained were different in each sub-district. For example, in Sentolo, Wates, Nanggulan, Temon, and Kokap sub-districts, corn plants were already in the generative phase at the time of sampling. This caused only *S. frugiperda* eggs to be found in those sub-districts. Meanwhile, in Pengasih, Kalibawang, Nang-gulan, Lendah, and Samigaluh subdistricts, only *S. frugiperda* larvae were found. This is because the corn plants were still in the vegetative phase at the time of sampling. In addition to these plant phase factors, the number of *S. frugiperda* eggs and larvae is also influenced by abiotic factors, such as air temperature, air humidity, and light intensity (Table 3).

The correlation test results show that the number of eggs is correlated with air temperature and air humidity, while there is no correlation with light intensity (Table 4). Based on the test results, the higher the air temperature, the higher the number of eggs, while the high air humidity will reduce the number of eggs.

Table 5. Correlation between the number of *S. frugiperda* larvae and abiotic factors

			Number of Individual Larva	Air Temperature	Air Humidity	Light Intensity
Spearman's rho	Number of Individual Larva	Correlation Coefficient	1.000	.179	-.306	.221
		Sig (2-tailed)	.	.414	.156	.310
		N	23	23	23	23
	Air Temperature	Correlation Coefficient	.179	1.000	-.776**	.156
		Sig (2-tailed)	.414	.	.000	.477
		N	23	23	23	23
	Air Humidity	Correlation Coefficient	-.306	-.776**	1.000	-.178
		Sig (2-tailed)	.156	.000	.	.415
		N	23	23	23	23
	Light Intensity	Correlation Coefficient	.221	.156	-.178	1.000
		Sig (2-tailed)	.310	.477	.415	.
		N	23	23	23	23

Note: ** Correlation is significant at the 0.01 level (2-tailed)

In contrast to the number of *S. frugiperda* larvae, the correlation test (Table 5) shows that the number of individuals of *S. frugiperda* larvae correlates with air temperature, humidity, and light intensity. Accordingly, high air temperature will also affect the number of *S. frugiperda* larvae, and high air humidity will also reduce the number of *S. frugiperda* larvae.

Attack Intensity of *S. frugiperda* in Corn Plants in Kulon Progo

This study also calculated the attack intensity level of S. frugiperda on corn plants in Kulon Progo. Based on this research, it is known that in 12 sub-districts in Kulon Progo, S. frugiperda attacks have been found. The attack intensity of S. frugiperda in Kulon Progo reached 4.97 %, classified as mild (Figure 2). Seasonal sampling-related issues were one of the factors that affected the result of this research. The sampling was done during the rainy season. Caniço et al. (2020) reported that S. frugiperda attacked a larger proportion of corn plants during the dry season than during the rainy season.

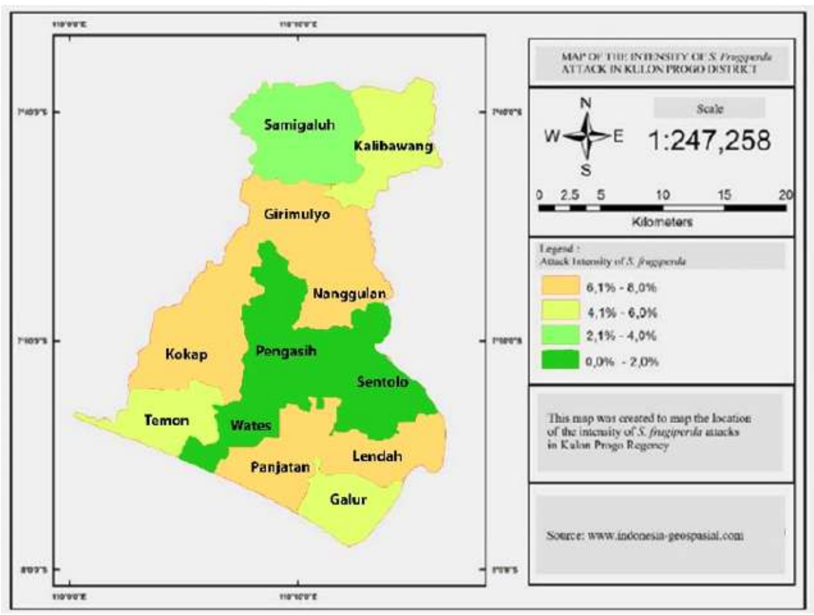

Figure 2. Map of attack distribution of S. frugiperda in Kulon Progo

Table 6. Attack intensity of *S. frugiperda* in Kulon Progo

Subdistrict	Attack Intensity (%)
Sentolo	1.85
Galur	5.14
Pengasih	1.88
Wates	1.5
Kalibawang	5.76
Nanggulan	8
Girimulyo	7.37
Lendah	6.82
Panjatan	6.33
Temon	4.48
Kokap	7.08
Samigaluh	3.79

Based on the results of the research conducted, the highest intensity of attack was found in Nanggulan (8 %), and the second highest was in Girimulyo (7.37 %) (Table 6). The intensity of the attack in Nanggulan could be caused by the fact that planting is not simultaneous. The lowest attack intensity was found in Wates (1.5 %). The low intensity of attack in Wates was influenced by the phase of the corn plant and the presence of alternative host plants (Uge et al., 2021). According to Trisyono et al. (2019), as the age of the plant increases, the level of damage to corn plants caused by S. frugiperda will decrease. Moreover, alternative host plants around corn fields can reduce the attack intensity of S. frugiperda (Wahyudin et al., 2018). This is in accordance with the statement of Asfiya et al. (2020), reporting that intercropping corn plants with several types of plants from the Fabaceae Family can reduce the level of S. frugiperda attack on corn plants.

CONCLUSION

The abundance of larvae on corn plants in Kulon Progo Regency was 12 individuals, consisting of five 2nd instar larvae and seven 3rd instar larvae. The attack intensity of *S. frugiperda* on corn plants in Kulon Progo Regency was 4.97 %, which is classified as low. *Spodoptera frugiperda* larvae in Kulon Progo Regency were found in four sub-districts, namely Kalibawang, Nanggulan, Pengasih, and Lendah.

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AUTHORS CONTRIBUTIONS

ILIP designed and conceived the experiments. ILIP and WHS conducted the experiment. ILIP, WHS, and YH contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by WHS. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Physiological Traits of Vanilla Plant (*Vanilla planifolia* Andrew) in Various Types of Shade Trees

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ABSTRACT

Vanilla plants are CAM species and are very sensitive to direct sunlight. Shade plants are crucial for mitigating the intensity of sunlight to protect vanilla plants. The physiological properties of vanilla plants under different shade trees were studied to understand how shade trees affect vanilla plants. The research applied a randomized completely block design with four groups. Planting was carried out using four shade plants, including *Gliricidia sepium, Syzygium aromaticums, Erythrina variegata,* and *Leucaena leucocephala*. Microclimate conditions such as temperature, humidity, and sunlight intensity were observed three times a day (morning, afternoon, and evening). Physiological observation parameters include chlorophyll A, chlorophyll B, total chlorophyll, proline, and relative water content. The research results showed that temperature, humidity, and intensity of sunlight varied depending on the type of shade plant. Types of shade plants significantly influence chlorophyll A content, chlorophyll B levels, total chlorophyll, proline, and relative water content. The conclusion indicated that the type of shade plant affects the microclimatic conditions of the vanilla plant, thereby influencing the physiological traits of the vanilla plant.

Keywords: Chlorophyll; Microclimate; Proline; Water Content

INTRODUCTION

Vanilla plant (*Vanilla planifolia* Andrews) is a tropical plant widely cultivated in Madagascar, Indonesia, China, and Mexico (Rahman et al., 2019). High concentrations of phytochemical elements with high antioxidant activities in stems and leaves showed that *Vanilla planifolia* is a source of antioxidants (Yusuf et al., 2023). Vanilla is a plantation commodity with high economic value (Mudyantini et al., 2024). Vanilla plants grow optimally at temperatures between 20 and 30 °C (Parada-Molina et al., 2022), with a minimum rainfall of 2,000 mm per year, sunlight intensity of 30 – 50 %, air humidity of 60 – 75 % (Iftikhar et al., 2023), and in the warmest, driest environment (Parada-Molina et al., 2022). The average rainfall of 2,050 and 2,070 mm gradually reduces the suitable growing area for vanilla species. There is a need to rearrange the vanilla agricultural production strategies by adopting sustainable and climate-smart practices and implementing a national strategy for conserving genetic diversity (Armenta-Montero et al., 2022).

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Vanilla plants are susceptible to direct sunlight, so they need a shade tree to reduce the intensity of sunlight. Excessive shading can destroy vanilla plants, significantly influencing the number of branches produced (Nugraha et al., 2024). Land and climate suitability, especially light intensity, are crucial factors, so the adjustment to land, climate, and cultivation techniques are necessary (Rosman, 2020).

The chlorophyll fluorescence, P700 redox state, and electrochromic shift signals were measured to examine photosynthetic characteristics under FL in *Vanilla planifolia*. The light use efficiency was high in the morning but was limited in the afternoon, indicating that the pool of malic acid dried down in the afternoon. During morning photosynthetic enhancement, electron flow through photosystem I quickly reached 95% of its maximum value within 4–6 min, indicating that V. planifolia exhibits a faster photosynthetic enhancement compared to C3 and C4 plants reported previously. Upon a sudden transition from dark to actinic light, *Vanilla planifolia* displayed a rapid re-oxidation of P700, indicating a swift outflow of electrons from PSI to alternative electron acceptors, which was attributed to the O2 photo-reduction mediated by the water-water cycle. The quantum photochemical yield of PSI and PSII of vanilla plants was mostly reduced in the afternoon compared to the morning. This suggests that CO_2 assimilation is very low in the afternoon. Meanwhile, the non-photochemical quantum in PSII and PSI increased markedly to protect PSI and PSII (Wang et al., 2022). The high relative humidity due to excessive shading decreased the yield of *Vanilla planifolia* (Andrade et al., 2023).

Vanilla plants do not require full sun (*shade-loving plants*). Therefore, protective plants are essential for vanilla cultivation. The diverse types of protective plants used for vanilla cultivation can significantly impact the intensity of sunlight the plant receives. The light intensity received by vanilla plants varied depending on the shading. Teak and jackfruit trees can be used as shading options in vanilla cultivation as they provide optimal sunlight intensity, resulting in 36% and 46% of yields, respectively (Nugraha et al., 2024). The vanilla plant is a CAM plant. High solar radiation can inhibit the process of photosynthesis and the growth of vanilla plants. However, in the long term, vanilla plants exhibit higher photosynthesis and growth at intermediate radiation levels (Sanchez, 2021). In CAM plants, stomata open at night when conditions are relatively cold and moist. PEP carboxylase operates at night, binding inorganic carbon to C_4 acid stored in large vacuoles. At noon, the stomata close to saving water, and the C_4 acid is decarboxylated to release CO_2, which is then fixed by Rubisco in the C_3 photosynthesis pathway.

Colored photoselective shade netting (CPSN) can alter the spectral light quality. The research result showed that red and blue CPSN affect the physiology traits and increase the antioxidant activity of *Vanilla planifolia*. Red CPSN can accelerate plant growth and biomass production, possibly due to its higher efficiency. However, further research is needed to know if red CPSN can also increase reproductive development and fruit yield (Sanchez, 2021). At an altitude of 825 m asl, vanilla plants produce better generative growth and yield components, although vegetative growth decreases. Vanilla orchids are not frost-tolerant and require tropical climatic conditions (Arya & Lenka, 2019). The flowering *of Vanilla planivolia* requires strong sunlight (Kitai & Lahjie, 2016). The research showed that blue light with a wavelength of 460 nm and red color light with a wavelength of 660 nm markedly improved stem elongation and chlorophyll synthesis of vanilla plants. Blue light markedly improves root elongation, the number of roots, and the number of leaves of vanilla plants (Ramírez-Mosqueda et al., 2017).

Vanilla plants can adapt to their light environments through morphological and physiological traits (Zhang et al., 2018). Changes in the absorption of sunlight by plants result in morphological and physiological changes. Leaf adjustment is achieved through filtration effect, light distribution, and adaptation to environmental conditions. The difference in the chlorophyll content between shaded and unshaded plants affects the density of photosynthesis photon flow. The transmission of light is carried out through vacuoles to shorten the distance that electrons must travel in the electron transfer chain. Physiological acclimatization is done by changing the chlorophyll arrangement and PSII/PSI ratio. Sheltered plants have a high PS II/PSI ratio and a/b chlorophyll ratio to increase the light-capture complex for more efficient photosynthesis (Yustiningsih, 2019). Shade conditions can improve gas exchange, reduce leaf temperature, and promote chlorophyll synthesis (Gómez-Bellot et al., 2023). This research aimed to determine the influence of types of shade trees on the physiological traits of vanilla plants.

MATERIALS AND METHODS

Research Area

The research was carried out on vanilla farms in Sinogo, Pagerharjo, Samigaluh, Kulonprogo from May to November 2023. The location was 7◦ 66 S, 110◦ 16 E, and 700 m above sea level.

Experimental Design

The research applied a randomized completely block design with 4 blocks. The treatment involves a type of shading plant, which includes 4 types of shade trees and climbing plants, namely *Gliricidia sepium, Syzygium aromaticum, Erythrina variegata,* and *Leucaena leucocephala.* The vanilla plants were 2 years old, with a planting distance of 80 x 150 cm. There were 5 plant samples for each different type of shade tree. The physiological parameters observed included chlorophyll A content, chlorophyll B levels, total chlorophyll, proline, and relative water content. Data analysis was carried out using analysis of variance (ANOVA) at the level of 5% and continued with the Duncan Multiple Range Test (DMRT) and correlation regression analysis. Daily measurements of sunlight intensity, temperature, and air humidity under the canopy of shade trees were taken for each plant sample at 6.00, 12.00, and 18.00.

Chlorophyll Content Analysis

Chlorophyll content was measured by sampling fully opened leaves. Chlorophyll was extracted from fresh leaves using 80% acetone filtered and then read with Spectronic 21 at wavelengths of 645 and 663 nm (Lichtenthaler, 1987).

Proline Content Analysis

Proline content was measured using a modified method (Bonjoch & Tamayo, 2001). The plant material for measurement was fully opened leaves. The leaves were mashed using a grinder, with 0.5 g of the material finely ground with a mortar with 10 ml of a 3% sulfosalicylic acid solution. The impact results were filtered with the Whatman 2 filter paper. A solution of ninhydrin acid was prepared by heating 0.50 g of ninhydrin in 30 l of glacial acetic acid and 29 ml of 6 M phosphoric acid until the solution was mixed. 0.5 ml of filtrate was reacted with 2 ml of ninhydrin acid in a test tube, then shaken and heated at 100 ^{0}C for one hour. The mixture was extracted with 5 ml of toluene and then cornered with a stinger for 15 seconds. After about 24 hours, the absorbent layer separated at the top and was aspirated with a pipette. The absorbent of the solution was read spectronic 21 D at a wavelength of 520 nm.

Relative Water Content Analysis

The relative water content of the leaves was determined by first measuring the fresh weight of the sample leaves, which were then saturated in water for 24 hours, after which they were weighed to obtain the turgid weight. After soaking, the samples were dried quickly with filter/tissue paper and immediately weighed to get a fully turgid weight (TW). The samples were then oven-dried at 80^{0}C for 24h and weighed to obtain dry weight (DW).

The relative moisture content (RWC) was calculated by the formula:

$$\text{RWC (\%)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100\ \% \quad (1)$$

RESULTS AND DISCUSSION

Microclimate of Vanilla Plants

Vanilla plants should employ shade to reduce the intensity of sunlight. The influence of shade plants varies across different parameters, including temperature, air humidity, and sunlight intensity (Table 1). The measurements of microclimate conditions revealed differences in temperature, air humidity, and sunlight intensity. Shading plants meet the requirements for optimal growing temperatures, as the temperatures range from 25 to 29 ^{0}C. The highest temperature was observed under the shade of *Gliricidia sepium*, while the temperatures under *Syzygium aromaticum, Erythrina variegata,* and *Leucaena leucocephala* shade plants were the same. Air humidity ranges from 65 – 75%, so the use of four types of shade met the requirements for growing vanilla plants. The intensity of sunlight under the shade of *Erythrina variegata* was 31%, under *Leucaena leucocephala* was 49%, under *Gliricidia sepium* was 59%, and under *Syzygium aromaticum* plants was 62%. This indicates that *Erythrina variegata* and *Leucaena leucocephala* plants are suitable shade plants for vanilla cultivation because they provide 30-50% of the sunlight intensity required by vanilla plants. This result is in line with (Rosman, 2020), showing that *Erythrina variegata* and *Leucaena leucocephala* trees qualify as suitable shading plants for vanilla.

Table 1. Microclimate data under various types of shade (± SE)

Types of Shade	Temperature (^{0}C)	Air Humidity (%)	Intensity of sunlight (%)
Gliricidia sepium	29 ± 0.53	75 ± 4.64	62 ± 0.10
Syzygium aromaticum	25 ± 0.80	67 ± 4.23	59 ± 0.13
Erythrina variegata	25 ± 0.62	65 ± 4.75	31 ± 0.09
Leucaena leucocephala	25 ± 0.53	67 ± 4.42	49 ± 0.09

The intensity of sunlight is a crucial factor that influences the growth and production of vanilla plants. During the vegetative phase, a lower intensity of sunlight is required compared to the generative phase. The intensity of the ray influences the temperature and humidity. There is a close relationship between the intensity of sunlight received by vanilla plants and both humidity and temperature. This is indicated by the results of correlation analysis, where there is a positive correlation between the sunlight intensity and air humidity (r = 0.712) and between the intensity of sunlight and temperature (r = 0.56). The results of the correlation analysis between temperature and humidity also showed a positive correlation (r = 0.977). This shows a close relationship between temperature and humidity.

Chlorophyll Content

Chlorophyll level is one of the main factors affecting the photosynthetic ability of plants. Using *Erythrina variegata* plants as the shade can significantly increase chlorophyll A, chlorophyll B, and total chlorophyll. There was a marked difference in the level of chlorophyll A, chlorophyll B, and total chlorophyll when comparing shading with *Erythrina variegata* to shading with *Leucaena leucocephala, Syzygium aromaticums* and *Gliricidia sepium* (Table 2).

Table 2. The effect of shade on the physiological traits of vanilla plants

Types of Shade	Parameters				
	Chlorophyll A (unit)	Chlorophyll B (unit)	Total Chlorophyll (unit)	Proline content (μmol/g)	Relative water content (%)
Gliricidia sepium	14.087 b	3670.191 c	26.203 c	0.066 c	20.899 b
Syzygium aromaticum	13.568 d	1885.965 d	22.254 d	0.157 a	13.797 d
Erythrina variegata	14.607 a	7484.309 a	31.910 a	0.059 d	29.163 a
Leucaena leucocephala	14.401 c	4999.367 b	28.542 b	0.073 b	20.825 c

Note: Means followed by the same letters in the column show a significance difference in the confidence level of 5%.

The chlorophyll A, B, and total chlorophyll levels were opposite the intensity of sunlight the vanilla plant received. The results of correlation analysis indicate a negative correlation between the intensity of sunlight and levels of chlorophyll A (r = - 0.787), chlorophyll B (r = - 0.917), and total chlorophyll (r = - 0.865). The intensity of sunlight under the shade of *Erythrina variegata* was 31%, under *Leucaena leucocephala* was 49%, under *Syzygium aromaticums,* was 59% and under *Gliricidia sepium* was 62%. This result is in line with previous studies on pineapple plants. Pineapple plants are CAM plants like vanilla, and high intensity of light in pineapple plants also reduces chlorophyll levels (Rodríguez-Escriba et al., 2015; Wang et al., 2020). Different research results are found in *Aralia errata*, a C_3 plant, where pigment photosynthesis of chlorophyll A, chlorophyll B, and total chlorophyll significantly decreases as shading increases or intensity of light decreases (reduction in chlorophyll content is directly related to the decrease in intensity of light) (Gao et al., 2019). Environmental factors such as light intensity affect the arrangement and role of the photosynthetic organs from C3 and C4 species (Hu et al., 2023). The results of this study are the same as research conducted by (Juhaeti et al., 2020), indicating that chlorophyll levels increase by 50% with 50% shading but decrease when shading reaches 75%.

The results showed that in vanilla plants, chlorophyll A levels and chlorophyll B levels significantly increased at low intensity of light (*Erythrina variegata* tree shade). The intensity of sunlight received by plants decreases when using shade trees with denser or larger leaves. *Erythrina variegata* plants have wider leaves, so the intensity of sunlight received by vanilla plants is lower. Low light intensity causes air temperature and humidity to decrease (Table 1). To increase the efficiency of light capture at low intensity, plants will reduce the light transmitted by increasing the number of chloroplasts and the pigment content within each chloroplast. According to (Ko et al., 2020), *Vanilla planifolia* is a loving plant. Vanilla chloroplasts started to decrease when blue light was higher than 20 µmol $m^{-2}s^{-1}$. Shade plants reduced leaf temperature and increased the synthesis of chlorophylls (Gómez-Bellot et al., 2023).

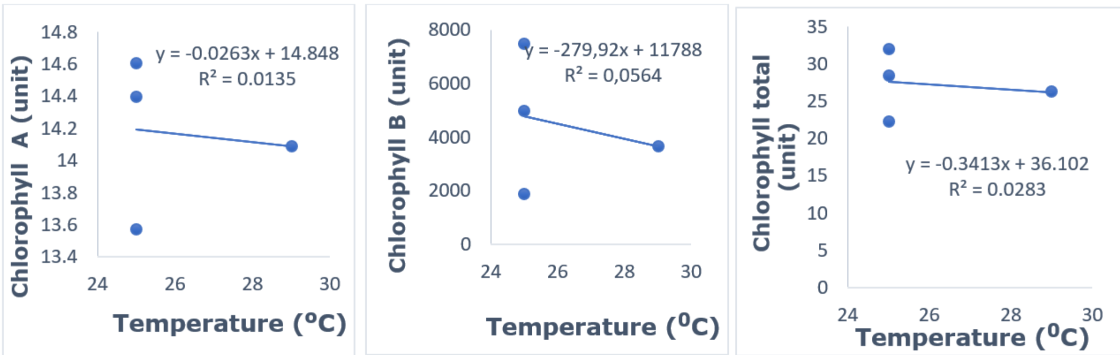

Figure 1. The correlation between temperature and content of chlorophyll A, chlorophyll B and total chlorophyll

The high intensity of sunlight increases the temperature. Increased temperature also decreases chlorophyll A, chlorophyll B, and total chlorophyll. This is shown by the results of regression analysis between temperature and levels of chlorophyll A (y = - 0.0263 x + 18.848), chlorophyll B levels (y = - 279.92 x + 11788), and total chlorophyll (y = - 0.3413 x + 36.102) (Figure 1). The results of this study differ from those of research on orchid plants, which are CAM plants. Most orchids, like vanilla plants, can adapt to their light environments through morphological and physiological traits (Zhang et al., 2018). Blue-red photoselective shade netting increased chlorophyll content and maximum quantum yield of photosystem II of the vanilla plant. In orchid plants, low temperatures further reduce chlorophyll A and chlorophyll B levels, while higher air temperatures lead to increased chlorophyll levels (Daems et al., 2022).

Proline Content

Proline is an osmolytic compound that helps plants resist drought stress (*drought tolerance*) through an osmotic adjustment mechanism. Light is a factor that greatly affects plants growth, development, and physiological processes. Plants can adjust their metabolism to environmental changes such as light by accumulating proline. When the intensity of light is very high, the accumulation of proline is three times greater than when the intensity is normal (Kovács et al., 2019). The results showed that *Syzygium aromaticum* shade obtained the highest levels of proline and significantly differed from *Erythrina variegata*, *Gliricidia sepium*, and *Leucaena leucocephala* shade. The intensity of sunlight received by vanilla plants was lower when under *Erythrina variegata* shade (31%), so that proline levels were lower. Conversely, the shade of *Syzygium aromaticum* resulted in a higher intensity of sunlight received by vanilla plants, leading to increased proline levels. High proline accumulation in response to increased temperatures was observed under the *Syzgium aromaticum* shade. According to (Raza et al., 2023), plants accumulate proline in response to temperature stress. There is a close relationship between the intensity of sunlight and proline levels. The results of the correlation analysis showed a positive correlation between the intensity of sunlight and proline levels (r = 0.484). According to (Zhang et al., 2022), free proline regulation is the main mechanism of plant adaptation to light stress. In CAM orchids, proline content in leaves increased after 7 weeks of drought under moderate light conditions (Tay et al., 2019).

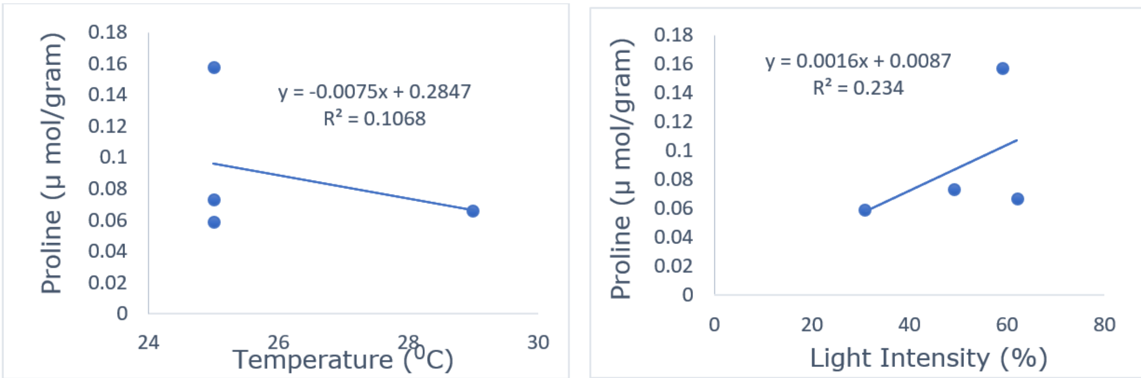

Figure 2. The correlation between temperature and light Intensity and proline Levels

The regression analysis results showed a positive linear regression between the intensity of light and proline levels (y = 0.0016x + 0.0087). This suggests that an increase follows an increase in the intensity of sunlight in proline levels. In contrast, a decrease in the intensity will be followed by a decline in proline levels (Figure 2). According to (Xie et al., 2023), slight shading significantly decreased the proline. On the contrary, plants accumulate proline in response to high-temperature stress (Raza et al., 2023).

An increase in air temperature, associated with higher light intensity, causes the proline levels of vanilla plants to decrease, even though the reduction is insignificant. This is shown by the regression analysis results between air temperature and proline levels (y = - 0.0075 x + 0.2847 (Figure 2). The study results are not in line with research on tomato plants, which are C3 plants where proline levels increase with higher temperatures (Suminar et al., 2021). This happens because C3 plants have a low saturation point for light intensity, leading to stress and increased proline levels as the intensity of light rises.

Relative Water Content

Relative water content is related to cell volume and can indicate a balance between the amount of water absorbed and that of water transpired. Plants that have a high relative water content are more resistant to drought stress. The results showed that the type of shade significantly affects the relative water content. The correlation analysis reveals a negative correlation between light intensity and relative water content (r = - 0.839), indicating that increased intensity of sunlight will reduce the leaf relative water content.

The regression analysis showed a negative linear regression between light intensity and relative water content (y = - 376.88 x + 40110). This shows that a decrease follows an increase in the intensity of light in relative water content, and conversely, a decrease in the intensity is followed by a rise in relative water content (Figure 3).

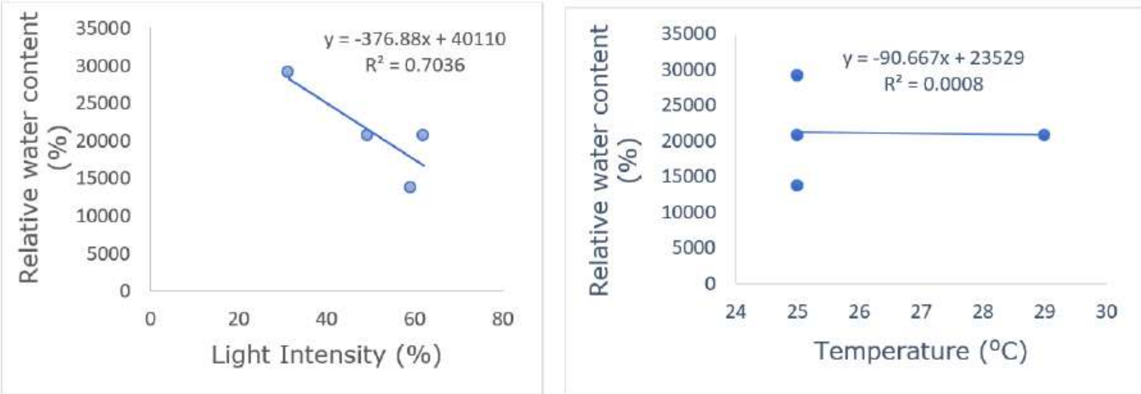

Figure 3. The correlation between light intensity and relative water content

In CAM plants with succulent leaves, such as vanilla plants, large vacuoles within the leaves enable them to store more water (Hu et al., 2023). It is a strategy of plants to store water in unfavorable conditions such as lack of water. The relative water content indicates the water status inside the plant. The higher the relative water content, the greater the amount of water stored in the tissues. The relative water content decreases under the conditions of lack of water (Arena, 2020). The intensity of sunlight received by vanilla plants under the shade of *Syzygium aromaticum* plants was 62%, and under *Gliricidia sepium* was 59 %. This condition can increase air temperature, leading to higher evaporation rates than under the shade of *Erythrina variegata*, consequently reducing the relative water content in the leaves. Conversely, under the shade of *Erythrina variegata*, the intensity of sunlight received by vanilla plants is low (31%), resulting in lower air temperatures, reduced evaporation, and higher moisture content in the leaves. The regression analysis shows these results between temperature and relative water content (y = - 90.667 x + 23539). The regression analysis shows the increase in air temperature causes the relative water content to be lower (Figure 3).

CONCLUSION

Erythrina variegata and *Leucaena leucocephala* are plants that qualify as shade and climbing trees for *Vanilla planifolia*. Using shading trees of *Erythrina variegata* increased chlorophyll A content, chlorophyll B levels, total chlorophyll, and relative water content but decreased vanilla plant proline levels. Higher intensity of sunlight decreases chlorophyll A, chlorophyll B, total chlorophyll, and relative water content, but high intensity increases vanilla plant proline levels.

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AUTHORS CONTRIBUTIONS

SS, HGM, and HO conducted the experiment. SS contributed to preparing the introduction and experimental design. HGM contributed to determined parameters. HO contributed to data analysis.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Quality Coefficient on Gene Differentiation and Phenotype: Clone Assessment of *Saccharum officinarum* Linn

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ABSTRACT

The production of superior sugarcane varieties can be achieved through crossbreeding between superior parent plants based on the desired advantages. Research examined the diversity of superior clones of SB04, SB11, SB19, and SB20 and identified the clones with the highest productivity potential. The first ratoon research was carried out from August 2020 to July 2021. Data analysis used descriptive-analytic methods, regression tests, and genetic diversity assessment. The observation was made on agronomic variables and potential productivity. Based on the result, the genetic diversity of the superior clone SB04 showed close similarity to the heterozygous combination PS862. The superior clone SB11 showed a tendency to inherit traits similar to Cenning. The superior clones SB19 and SB20 lean towards the VMC71-238 variety and the combination of PSBM01 and VMC71-238. The superior clones SB04, SB11, SB19, and SB20 produced higher weight, yield, and sugarcane crystal content compared to the characteristics of their two parents. Clones SB04, SB11, SB19, and SB20 produced high crystal content, ranging from 8.47 to 15.26 tons/ha, higher than their parent plants. SB19 had the highest yield, namely 15.26 tons/ha. Although some clones dominate crystal production, other clones inherit traits from both parents but are less dominant in overall productivity.

Keywords: Clone; Coir levels; Gene differentiation; Productivity; Superior

INTRODUCTION

Sugarcane, a nutritious plant from the grass family, is fresh and easy to cultivate on a wide scale (Bhatt, 2020; Rosales, 2021). It is regarded as one of the world's premier food crops (Ali et al., 2019). It has become a significant industrial commodity in Indonesia in the pursuit of sugar self-sufficiency. According to the Directorate General of Plantations, sugarcane production demonstrated a decreasing trend from 2015-2019, with yields of 2,497,997 tons, 2,204,619 tons, 2,121,671 tons, 2,174,400 tons, and 2,450,000 tons, respectively (Uchtiawati et al., 2020).

The primary factor contributing to low sugar productivity in Indonesia is the scarcity of high-yielding sugarcane varieties (Sulaiman et al., 2019). Most superior varieties cultivated by farmers mature slowly, leading to an imbalance in the optimal composition of plant maturity (Pathirana et al., 2020). The low sugar productivity in Indonesia results in a decrease in potential productivity at

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harvest. The suboptimal maturity of harvested sugarcane is due to the simultaneous planting of early, middle, and late-ripening varieties (Calderan-Rodrigues et al., 2021). Such conditions can lead to a decline in productivity, particularly yield, because the optimum level of maturity inherited genetically varies across varieties. Consequently, the potential yield for sugar also differs (Cursi et al., 2022). Environmental factors can also influence low potential productivity (Montaldo et al., 2021; Neto et al., 2020). Insufficient water supply, salt stress, high temperatures, low humidity levels, and nutrient deficiencies can all decrease the potential productivity of high-yielding sugarcane varieties.

This critical and intricate issue in the sugarcane-based industry necessitates intensive, comprehensive, and sustainable research (Restu et al., 2021; Amna et al., 2020). The productivities of sugarcane should be complemented by government regulations that promote the growth and development of a healthy, honest, fair, modern, and globally competitive sugar industry. The breeding and collection of high-yielding varieties can enhance the quality and quantity of sugarcane production (Ali et al., 2019; Hamida & Parnidi, 2019; Restu et al., 2021). The production of superior varieties can be achieved through cross-breeding between superior parent plants based on desired advantages. Cross-breeding between clones can increase the diversity of F1 progeny to form new clones. Additionally, cross-breeding can be conducted between superior varieties and clones based on the desired potential superiority. The expectation is that the resulting offspring or F1 or clones will exhibit high productivity potential and resistance to pests and diseases.

Cross-breeding in previous studies has yielded several superior clones with high potential productivity, resistance to pests and diseases, and high coir content (Anwar et al., 2021; Prabowo et al., 2021). These clones include SB01, SB03, SB04, SB11, SB12, SB19, and SB20. To date, these seven clones have undergone superiority testing in multiple locations, including Plantcane and Ratoon One, as well as in the superiority test of Ratoon Two. Each variety of plant species possesses specific morphological descriptions, and numerous publications have explored the identification of varieties based on morphological characteristics (Tesfa et al., 2024). Newly created superior varieties require periodic review at least every 10 years due to the potential decline in resistance of commercial varieties to new pests and diseases and the emergence of more productive varieties (Begna, 2020).

By relevant regulations, the assembly of various stages of selection and superiority tests serves as the benchmark for producing new high-yielding sugarcane varieties (Heliyanto & Abdurrakhman, 2022). Clones that pass the selection and multi-location productivity potential tests and demonstrate resistance to pests and diseases can be submitted to the Minister of Agriculture of the Republic of Indonesia by the rules stipulated in the online application. The superior clones that are created and produced must possess both special and general advantages over existing superior varieties and embody the character and productivity potential of the two parent plants. These special and general advantages include superior sugarcane weight per hectare, yield, and crystal content per hectare, resistance to pests and diseases, and high coir content (Heliyanto & Abdurrakhman, 2022).

Genes govern quantitative variables related to morphological and physiological characteristics and result from growth processes. These variables are regulated by several genes, referred to as multiple genes or polygenes (Napier et al., 2023). Each gene exerts a minor effect, while environmental influences have a substantial impact (Montaldo et al., 2021). Quantitative variables can be measured using specific units of measure and are controlled by many genes, thus characterizing them as polygenic characters (Meena et al., 2022). Each gene unit has a minor influence on expressing the phenotype, which is termed a minor gene (Das & Bansal, 2019). The selection of quantitative variables can be conducted based on statistical data. Data testing involves calculating the mean, variance, and standard deviation (Mertler et al., 2021).

Qualitative variables are typically discrete, reflecting the observed plant phenotype, and can be visually distinguished (Uchtiawati et al., 2020). One or more genes regulate these variables (Anwar et al., 2021). If controlled by a single gene, it is referred to as a monogenic character, and if controlled by several genes, it is termed an oligogenic variable (Campuzano et al., 2020). Each gene plays a significant role in expressing the phenotype and is a major gene (Zhang et al., 2021). These variables are differentiated based on the presence or absence of environmental influences. Data collection for these variables is conducted through observation techniques (Reckling et al., 2021).

Qualitative variables serve as the primary characteristics of a species due to their minimal environmental influence and ease of transmission to offspring (Komape, 2019). Selection activity is deemed effective if the original population has two conditions: substantial phenotypic diversity and a sufficiently high heritability value (Saini et al., 2020). The effectiveness of selection activities on a character increases with the heritability value of that character (Tena et al., 2023). While there is no standard heritability value, several scientific journal articles suggest that a heritability value is considered low if it is less than 20 %, moderately high if it is between 20-50 %, and high if it is greater than 50 % (Kumari et al., 2020; Netoet al., 2020; Prabowo et al., 2021). These values are highly dependent on the method and population studied.

Breeders often regard the purported genetic progress as a percentage above the average value of the population (Snowdon et al., 2021). The genetic aligns with the explanation provided by the previous finding that Genetic Gain (KG) (%) is the product of differential selection values and heritability, which determines the efficiency of the selection system. Consequently, selection will be effective if a high genetic progress value is supported by one of the high heritability values (Barreto et al., 2019), resulting in high heritability.

This study has identified variations in crystallization productivity among sugarcane clones, but there is still a gap in the deeper understanding of the genetic mechanisms underlying this quality differentiation. While previous studies have focused more on identifying superior clones based on agronomic outcomes, this study needs to explore further how specific interactions between genes and the environment contribute to different phenotypic expressions. In addition, further studies are needed on how epigenetic factors influence the stability and inheritance of these superior traits in subsequent generations to ensure continued high productivity and adaptation to changing environmental conditions.

MATERIALS AND METHODS

Study area

The study was carried out from August 2020 to July 2021 at the Center for Sugar Cane Research and Development (P3T) at the Faculty of Agriculture, University of Muhammadiyah Gresik, in collaboration with Gempolkrep PG PT Perkebunan Nusantara X (PTPN X) in Sambiroto Village, Sooko

District, Mojokerto Regency. Geographically, Mojokerto Regency is situated between 111°20'13" to 111°40'47" east longitude and 7°18'35" to 7°47'0" south latitude. Sooko district is located at an altitude of 64 meters above sea level with an average air temperature of approximately 29.8°C and air humidity ranging from 74.3 - 84.8 %. The soil type at the site is alluvial, containing N content of 0.11 %, P_2O_5 of 30 ppm, K_2O of 30 ppm, K_2O of 154 ppm, organic C of 1.08 %, organic matter of 1.86 %, pH of 5.79, and moisture content of 0.29. The average rainfall recorded in Sooko District in 2018 was 227 mm.

Description of Clones

The clones of sugar used were SB04, SB11, SB19, and SB20 clones. Ratoon cane 1 underwent maintenance in August 2020 and was harvested in July 2021. The data on hablur (sugar) is presented in Table 6. The four superior clones were planted in a plant cane manner in July 2019 and harvested in August 2020. The crystal content was very high, with values of 9.41 tons/ha, 10.76 tons/ha, 11.63 tons/ha, and 8.92 tons/ha, respectively. Ratoon cane 2 was harvested in August 2022. The hablur (sugar) produced by ratoon cane 2 was very high. The crystals produced had yields of 10.05 tons/ha, 9.73 tons/ha, 10.21 tons/ha, and 11.18 tons/ha, respectively.

Methodology and Data Analysis

The data about the four clones were analyzed using descriptive, analytical, diagnostic, and differential diagnostic methods. Analytical descriptions encompass all the natural traits or characters of the organism (natural character), while diagnostic descriptions contain only important traits (essential traits), which serve as distinctive identifiers. Differential diagnostic descriptions distinguish sugarcane clones from each other by mentioning the different varieties being compared. The description guidelines adhere to PP No. 19 of 2021 concerning Genetic Resources and the Release of Plantation Plant Varieties, PP No. 38 of 2019 concerning the Release of Plant Varieties, and Ministerial Decree No. 1/KPTS/Kb020/1/2018 Amendment to the Decree of the Minister of Agriculture No. 318/KPTS. Kb,020.10.2015 concerning Guidelines for Production, Certification, Distribution, and Supervision of Sugarcane Seeds.

The research utilized both qualitative and quantitative analysis methods. Quantitative variables, which are governed by genes and pertain to morphological and physiological traits, were measured, including stem height, stem diameter, yield, crystal content, and harvest weight (Patra, 2022). Conversely, qualitative variables are discrete and visually observed to distinguish plant phenotypes. One or more genes also control these variables and can be either monogenic or oligogenic. The presence or absence of environmental influences distinguishes these variables. Data collection was conducted through observation techniques. The research design for the ratoon cane was a randomized block design (RBD) consisting of six clones (SB04, SB11, SB19, SB20, PS881, and BL) as treatments and three replicates. The experimental plot size was 10 x 8 meters. The pedigree selection was conducted in 2013, and the clones underwent several laboratory tests for excellence, including Green House, Pot trial, and Polybags. Furthermore, the clones underwent preliminary and multi-location tests in several dry land typologies, paddy fields, and agro-climates until 2018. Productivity tests were conducted in multi-locations plant cane in 2019, followed by ratoon cane one in 2020 and ratoon cane two in 2021. The data were analyzed using ANOVA with a 5 % LSD test, regression analysis, genetic diversity assessment, and analytical description.

Genetic Diversity and Regression Test

The regression value is utilized to ascertain the extent of influence that several independent variables exert on the dependent variable, and the values of the dependent variables can be a predictor if all the independent variables have known values. Regression analysis is employed to predict the value of one or more response variables (dependent variables) based on several predictor variables (independent variables). If x1, x2, . . ., xp are predictor variables with p variables that have a relationship with a response variable Y, then a general linear regression model with one response variable can be represented as follows:

$$Y = a + b1X1 + b2X2 \quad (1)$$

Y = dependent variable (variable value to be predicted)
A = constant
b1,b2,…, bn = regression coefficient value
X1,X2,…, Xn = independent variable

Genetic diversity is a variation within a population due to diversity among individuals as members of the population (Filho et al., 2021).

$$\sigma^2 g = (M3 - M2) / rl \quad (2)$$

$$\sigma^2 gxe = (M2 - M1) / r \quad (3)$$

$$\sigma^2 e = M1 / rl \quad (4)$$

$$\sigma^2 p = \sigma 2 g + \sigma 2 gxe / l + \sigma^2 e \quad (5)$$

and

$$KKG = \frac{\sqrt{\sigma^2 g}}{\bar{x}} x\ 100\% \quad (6)$$

The breadth or narrowness of the genetic diversity value of a trait is determined based on the genetic variance and standard deviation of gene variance according to the following formula:

$\sigma^2 g > 2\ \sigma\sigma^2 g$: broad genetic diversity,

$\sigma^2 g < 2\ \sigma\sigma^2 g$: narrow genetic diversity (Tolera et al., 2023).

RESULTS AND DISCUSSION

Kinship

Grouping analysis to determine the relationship between the sugarcane varieties studied was carried out based on the morphological traits description in Figure 1.

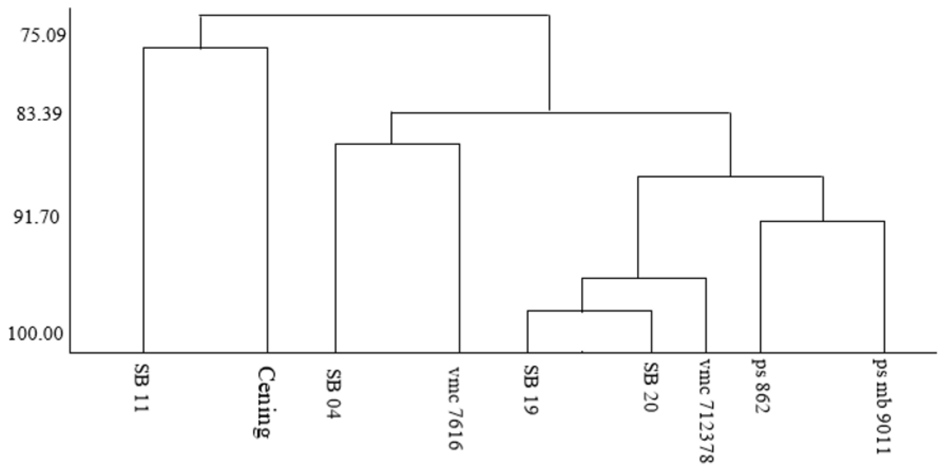

Figure 1. Dendrogram of the Relationship between Sugarcane *(Saccharum officinarum* Linn*)* Clone SB04, Clone SB11, Clone SB19, Clone SB20, and the Parental Varieties

Figure 1 depicts two groups or clusters of sugarcane varieties *(Saccharum officinarum* Linn*)* based on their morphological traits, with a similarity value of 75.09 %. Group I comprise sugarcane varieties Cenning and SB 11, while Group II includes varieties VMC 76-16, PSBM901, PS862, VMC71-238, and clones SB04, SB19, and SB20. The agglomerative coefficient values indicate the degree of similarity between sugarcane varieties. Clones SB04, SB19, and SB20 exhibit a closeness of 88.85 to variety VMC711 but not to clone SB11 or variety Cenning.

Genetic Diversity, Potential Productivity, and Coefficient of Genetic Diversity

The diversity value for quantitative variables can be determined based on the coefficient of genotypic diversity (GST) and the coefficient of phenotypic diversity (PST). These values ascertain the potential for selection progress for the trait under examination. The coefficient value of genetic diversity is presented in Table 1.

Table 1. The Coefficient Value of Genetic Diversity (GST) and Coefficient of Phenotypic Diversity (PST)

CHARACTER	GST (%)	PST (%)	Adms
Stem Height	29.181	29.297	Slightly Low
Rod Diameter	0.092	0.100	Low
Number of segments	2.204	2.430	Low
Brix	2.866	2.902	Low
Sugarcane Weight	104.745	231.351	hight
Yield	0.356	0.361	Low
Crystal	9.773	21.802	low

All the traits observed in this study exhibited high heritability estimates, except the percentage of stem weight and crystal content. A high heritability value signifies a strong correlation between phenotype and genotype. In this context, genetic factors exert a greater influence than environmental factors on the manifestation of the trait or phenotype. The environmental influence suggests that the action of additive genes is highly prominent, as proposed by Yadav et al. (2023). Most traits display broad genetic and phenotypic diversity, as evidenced by the diverse GST and PST scores. High diversity ensures the effectiveness of the selection made on the population.

As per the data presented in Table 1, the clones SB04, SB11, SB19, and SB20 exhibited high genetic diversity values in a broad sense. A higher GST value indicates a greater potential for successful technical culture improvement across various multi-location productivity potential tests for these clones in dry land and rice fields, thereby leading to optimal productivity.

A high GST value also results in a wider variability of traits, which can subsequently enhance genetic progress. A large coefficient of genetic diversity suggests that genetic manipulation of a trait with that coefficient has a high probability of success. Conversely, a trait with a small coefficient of genetic diversity will have a lower likelihood of successful improvement (Luo et al., 2022).

The clones SB04, SB11, SB19, and SB20 exhibited high PST values. A high FCF value suggests that environmental factors significantly influence the observed diversity. If the PST value is low and the GST value is high, it implies that genetic factors predominantly influence diversity. Conversely, a high PST value and a low GST value indicate that environmental factors largely influence diversity.

The observed high phenotypic diversity can be attributed to substantial environmental diversity (Zan et al., 2020). This high phenotypic diversity is due to significant environmental and genetic diversity resulting from segregation (Khan et al., 2021). The displayed diversity is the phenotype resulting from differences in genotypes.

A low genetic variance value in a population results from environmental variance exceeding phenotypic variety (Tolera et al., 2023). The absence of genetic variance does not mean that no genes affect the appearance of the diversity of clones SB04, SB11, SB19, and SB20. However, these genes are only expressed because dominant environmental factors cover them.

The yield diversity trait of clones SB04, SB11, SB19, and SB20 exhibited relatively high GST and comparatively low PST values. However, the crystal content and stem weight trait demonstrated high values for both GST and PST. The diverse traits of these clones displayed narrow variability. This narrow variability suggests that each individual in the population tends to be homogeneous, reducing the likelihood of obtaining a superior new generation (Hoarau et al., 2022). The high GST and PST values were particularly evident in the crystal content and stem weight traits. Despite both coefficients of diversity yielding high values, the PST value surpassed the GST value. This indicates that environmental factors exert a greater influence on the diversity of crystal content and stem weight in sugarcane clones SB04, SB11, SB19, and SB20.

Based on empirical evidence, the execution of multi-location tests to evaluate the potential productivity of plant cane and ratoon clones SB04, SB11, SB19, and SB20 in dry land and paddy fields is essential for determining their optimal productivity potential and stability.

The analysis of quantitative variables for clones SB04, SB11, SB19, and SB20, as presented in Table 1, revealed high GST values for the crystal content and stem weight per hectare. The yield trait exhibited a relatively high GST value. The crystal content and stem weight per hectare traits demonstrated a high FCF value, while the yield character showed a relatively low PST value. The genetic diversity analysis indicated high GST values, particularly for the crystal content and stem weight per hectare traits. The observed traits had KK values ranging from 27-28262 %. A greater coefficient of diversity on observed traits suggests a wider distribution of clone data (Ahmed et al., 2019; Alarmelu et al., 2021). The environment exerts varying influences on the observed variables. The Coefficient of Genetic Diversity (GST) and Phenotype (PST) are expressed in percentages (%). The GST and PST values for yield traits were 27 % and 65 %, respectively; for crystal content, they were 28262 % and 266 %; and for stem weights, they were 9536 % and 117%. Most GST and PST calculations displayed high criteria, while the yield trait showed relatively high criteria.

These results suggest that productivity in terms of crystal content and weight of sugarcane per hectare is high and is influenced more by genetic factors than environmental factors and vice versa. The high and low PST values describe the diversity of the visual traits of the four superior clones tested.

The genetic diversity of the four clones (SB04, SB11, SB19, and SB20), when planted in the limited environmental conditions of Mojokerto's alluvial dry land, exhibited varied responses. The observed differences in the genetic diversity of each clone were consistent with the traits of their parent varieties (Khan et al., 2022). The findings of this study highlight the suitability of each clone to its respective growing area typology, thereby contributing to an increase in productivity potential, particularly in sugarcane weight, yield, and crystallization optimization. Each clone aligns with Barreto et al.'s assertion that qualitative traits can be visually discerned (Barreto et al., 2021), and that these qualitative characteristics remain relatively stable despite changing environmental conditions (Xu et al., 2021). The qualitative trait of each clone plays a crucial role in germplasm conservation. The genetic diversity trait of each clone is manifested in the four superior clones selected and tested for their superiority. This ensures that the superior clones that pass the selection and have been tested for superiority are distinct and can be differentiated from one another.

The results of the genetic diversity test for each clone corroborate previous studies, which stated that there were trait differences between sugarcane clones, as evidenced by examining the morphological traits of each clone (Ahmed et al., 2019; Hamida & Parnidi, 2019). This study demonstrated that each clone could develop unique traits but tended to thrive in dry environmental conditions in Mojokerto's alluvial soils.

Potential Productivity Analysis

The potential advantages of the SB04 clone's productivity are presented in Table 2, showing that the productivity value of SB04 was higher than its parent, namely the PS 862 variety. The SB04 clone tends to inherit the trait description of the PS 862 parent.

Table 2. Comparison of SB04 Clone Production with its Parents Polycross

Varieties	Variable			
	Yields (%)	Crystal (ku/ha)	Rod Weight (ku/ha)	Maturity
SB04	9.25	134.12	1450	Middle–Slow
Tetra (PS 862)	9.45±1.51	91.0±16.3	993±1.02	Early-Middle

SB11 clone has high potential productivity compared to its parents, as shown in Table 3. As indicated in Table 3, the SB11 clone demonstrated higher potential productivity than the Cenning parent across all variables, although it did not surpass the VMC76-16 parent. The SB11 clone tends to inherit the descriptive trait of a.

Table 3. Comparison of SB11 Clone Production with its Parents

Varieties	Variable			
	Sugar Content (%)	Crystal (ku/ha)	Rod Weight (ku/ha)	Maturity
SB11	8.63	80.47	933	Slow
Parent (VMC76-16)	10.02±0,52	88.27±19.9	1105±182	early-middle
Parent (Cenning)	10.97	71.14	775	early-middle

The SB19 clone has high potential productivity, which is presented in Table 4. As indicated in Table 4, the SB19 clone demonstrated higher potential productivity than the VMC71-238 parent across all assessment criteria, although the yield value was not superior to that of the parent. The SB19 clone tends to inherit the descriptive trait of the parent.

Table 4. Comparison of SB19 Clone Production with its Parent

Varieties	Variable			
	Yield (%)	Crystal (ku/ha)	Rod Weight (ku/ha)	Maturity
SB19	11.25	152.62	1650	Early
Parent (VMC71-238)	10	110	1100	early-middle

The SB20 clone exhibited high potential productivity, as presented in Table 5. As indicated in Table 5, the SB20 clone demonstrated higher potential productivity than both parents across all variables, except for the yield value, which was lower than the parent's yield. The SB20 clone tends to inherit the descriptive trait of VMC 71-238.

Table 5. Comparison of the Production of SB20 Clones with their Parents

Varieties	Variable			
	Sugar Content (%)	Crystal (ku/ha)	Rod Weight (ku/ha)	Maturity
SB20	8.14	125.36	1540	Early
Parent (VMC71-238)	10.02±0.52	88.27±19.9	1105±182	early-middle
Parent (PSBM-901)	9.93±1.002	69.5±16.3	704±162	early-middle

The analysis presented in Tables 2 to 5 reveals that the four clones (SB04, SB11, SB19, and SB20) tend to inherit morphological traits from their respective parents. Kinship mapping can be utilized to determine the diversity of germplasm for plant breeding purposes. The SB04 clone inherits traits from its heterozygous polycross parent, PS 862, and exhibits a tall stem stature but is susceptible to collapsing in strong winds. It has a yield potential of 13.41 tons/ha. The SB11 clone inherits a trait from Cenning and features a dense and tall clump stature with straight stems exhibiting reddish cylindrical segments. The stems have a waxy coating, but it does not affect their color. The yield productivity potential is 8.04 tons/ha.

The SB19 clone inherits characteristics from VMC 71-238 and has an upright stature with straight stems featuring green cylindrical segments with a thick wax coating. It has pest and disease-resistance properties and a yield productivity potential of 15.26 tons/ha. The SB20 clone inherits traits from the combination of its parents, VMC71-238 and PSBM 901, with a yield potential of 12.53 tons/ha. These percentage values for the morphological characters and productivity can serve as a reference for genetic studies and kinship analysis between the four tested clones, which are deemed superior for alluvial soils in Mojokerto and multiple locations.

The four superior clones under investigation exhibited variations in their physical traits. These variations were attributed to the inheritance of morphological traits from their parent varieties and their predisposition towards specific parent varieties. The morphological included agronomic potential and sugarcane production (Cursi et al., 2022). Qualitative traits are defined as traits that exhibit qualitative differences and can be easily categorized (Tesfa et al., 2024). Simple genes typically govern these traits (Budeguer et al., 2021). This analysis aimed to ascertain the genetic relationship among the four clones (SB04, SB11, SB19, and SB20) in a quantifiable manner. Regression analysis indicated that the observed values were a consequence of genetic and environmental influences.

The productivity of these clones was found to be optimal under environmental conditions conducive to their growth. These four superior clones (SB04, SB11, SB19, and SB20) are undergoing further testing to evaluate the stability of their productivity across multiple locations with varying soil types, including vertisol, alluvial, regosol, and grumusol. Despite these ongoing tests, their productivity continues to surpass that of older varieties and controls.

This study aimed to investigate the productivity potential of superior clones SB04, SB11, SB19, and SB20 in the Sambiroto Mojokerto plantation when planted as plant cane instead of ratoon cane. The study was conducted on dry land with a 75 % conversion per hectare and one type of alluvial soil. The results of the 5 % LSD test are presented in Table 6.

Table 6. Productivity Potential of Plant cane and Ratoon cane one Clone SB04, SB11, SB19, SB20, Conversion 75%, LSD 5%

Varieties	Plant Period	Harvest Time	Ripeness	Productivity				
				Brix (%)	Weights (q/ha)	Yield (%)	Crystal (ton/ha)	Conversion 75 %/ Ha (ton/ha)
Plant Cane								
SB04	07B 2019	08B 2020	Middle-slow	21	1256	7.49	9.41	7.32 a
SB11			Slow	23	1223	8.80	10.76	8.72 ab
SB19			Preliminary	20	1473	7.90	11.63	8.92 b
SB20			Preliminary	22	1130	7.90	8.92	7.52 a
PS 881			Preliminary	22	1216	9,06	11.02	8.27 a
BL			Slow	19	1264	7.52	9.51	7.13 a
LSD 5%								1.33
Ratoon Cane 1								
SB04	08B 2020	07B 2021	Middle-slow	25	1450	9.25	13.41	11.60 b
SB11			Slow	22	933	8.63	8.04	10.93 ab
SB19			Preliminary	24	1650	9.25	15.26	11.63 b
SB20			Preliminary	22	1540	8.14	12.53	9.90 ab
PS 881			Preliminary	22	1360	9.30	12.64	10.50 b
BL			Slow	20	1363	8.53	11.62	9.73 a
BNT 5%								2.49

Note: In the same column followed by different letters indicates significantly different based on the 5% LSD test.

Table 6 shows the potential productivity of clones SB04, SB11, SB19, and SB20 by plant cane and one type of alluvial soil.

As per the analysis in Table 6, the SB04, SB11, SB19, and SB20 clones exhibited high productivity potential. However, the productivity potential of each clone varies when evaluated based on indicators such as sugarcane weight, yield, and crystal production per hectare (Mehdi et al., 2024).

The disparity in the potential productivity of each clone can be attributed to the differing potential productivity inherited from each parent. This affects metabolic processes, particularly cell division, enlargement, and elongation (Pocovi et al., 2020). The process of converting sap into sucrose also varies. The converting aligns with the findings of (Ahmed et al., 2019; Kumari et al., 2020), which suggest that the productivity potential of superior varieties is not uniform.

In 2013, a plant breeding process was conducted at the Perning Mojokerto field using parent plants, including PL 55, Cenning, BM 90-1, VMC.71.238, BM 90-1, VMC 76-16, BL, and PS 862. The goal was to produce new superior varieties based on genetic diversity and breeder preferences (Anwar et al., 2021). The Hablur clones SB04, SB19, and SB20 demonstrated higher productivity than their parent plants and the PS 862 variety and exhibited early to mid-maturity. Clone SB11 produced a higher crystal content than its parent plants and the slow-ripening Bululawang variety. However, a 5 % LSD test did not reveal a significant difference compared to the other clones.

The crystals produced in this study were minimal, as the conversion rate was 75 % of the one-hectare area (Table 6). These results are consistent with previous findings (Heliyanto & Abdurrakhman, 2022) (3). Interestingly, one of the clones possesses a unique feature of feather covering on its back, which is around 20-25 % between the segments of the stem. (4) They are well-adapted for cultivation in the D3 agroclimatic type, characterized by alluvial soil topology in dry Raton cane 1 land. These clones display both early and late maturity types and have the potential to produce high fiber content while being resistant to fire blight disease. (5) Future research should extend to determining productivity superiority in different agroclimatic conditions, such as C2 and C3 types, and soil typologies, including chromosols, vertosols, and podzolic. DNA testing should also be conducted to ascertain the precise reasons for the high productivity traits. Furthermore, resistance to fire blight disease and fiber content testing should be carried out in various settings. (6) The crystal content and stem weight traits had a high coefficient of gene differentiation (Gst) and phenotypic differentiation coefficient (Pst), namely crystal content of 28262 % and 266 % and stem weight of 9536 % and 117 %, respectively. The four superior clones have the advantage of high crystal content, resistance to smut disease, and high coir content, which are potential renewable bioenergy sources.

The regression value is utilized to ascertain the extent of influence that several independent variables exert on the dependent variable. It can also predict the value of the dependent variable if all the independent variables have known values. The results of the regression test are presented in Table 7.

Table 7. Regression Value

Predictor	Coef	SE	t stat	p-value
Constant	0.335	2.202	-0.15	0.887
X1	0.39223	0.09475	4.14	0.014
X2	0.00032	0.000247	1.29	0.265
S	0.2553			
R-Sq	81.10%			
R-Sq(adj)	71.60%			
Sig. F	0.048			

This study used the multiple linear regression equation model: $Y = a + b_1X_1 + b_2X_2$. By examining the regression model and the results of the multiple linear regression, we can derive an equation that represents the factors influencing sugarcane productivity. This equation provides a mathematical representation of how various factors contribute to the overall productivity of sugarcane.

$Y = 0.33 + 0.392X_1 + 0.00032X_2$. As indicated in Table 7, the calculated F value is 0.335, with a significance level of 0.048. The results of analysis suggests that the probability is less than the tolerable significance level (0.048 < 0.05). Consequently, the yield variable has a significant positive effect on clone productivity. The coefficient of determination is utilized to determine the percentage of the effect of the yield variable on productivity. According to the regression test results in Table 7, the coefficient of determination (R square) is 81.10 %. This demonstrates that productivity is influenced by internal factors by 81.10 %, and external factors influence 18.9 %.

Based on the analysis presented in Table 7, the four superior clones (SB04, SB11, SB19, and SB20) exhibited high productivity. The superior clones was substantiated by the productivity data from two planting periods, namely the plant cane (PC) and the ratoon cane I (RC1), which were analyzed using multiple linear regression analysis. Sugarcane production is influenced by several factors, such as the fact that it does not operate independently and affects productivity. The production components utilized were data on sugarcane stalk production per planting level and resulting sugar production data, which were analyzed using correlation analysis. The research analysis results support the first hypothesis that variable (X1) has a partially positive effect on crop production, as indicated by the regression coefficient X1 of 0.392. Essentially, every unit increase in a variable (X1) will enhance productivity by 0.392 units. Variable (X2) also has a partially positive effect on plant productivity, as indicated by the regression coefficient X2 of 0.00032. The variable analysis implies that every

Table 8. Resistance to Fire Wounds and Coir Content of Superior Clones SB04, SB11, SB19, SB20 in Plant cane and Ratoon Cane One

Varieties	Plant Period	Harvest Time	Observation Attack (%)	Inoculation Attack (%)	Coir rate (%)	POL (%)
Plant Cane (Corsample)						
SB04	07 B 2019	08 B 2020	0.58	-	15.25	10.68
SB11			0.00	-	16.30	11.30
SB19			0.00	-	16.95	11.05
SB20			0.00	-	17.20	11.76
PS 862				-	-	-
BL				-	-	-
Ratoon Cane 1(Laboratory)						
SB04	08 B 2020	07 B 2021	0.58	-	14.80	-
SB11			0.00	-	15.81	-
SB19			0.00	-	16.45	-
SB20			0.00	-	16.23	-
PS 862				-	13.47	-
BL				-	13-14	-
Plant Cane (Polybag) age up to 6 months						
SB04	08 B 2022	02 B 2023	-	7.41	-	-
SB11			-	7.41	-	-
SB19			-	3.70	-	-
SB20			-	3.70	-	-
PS 862			-	14.81	-	-
BL			-	22.22	-	-

unit increase will augment crop production by 0.00032 units. A probability value smaller than 5 % (0.048 < 0.05) indicates that variables X1 and X2 positively increase sugarcane productivity. The results of this analysis illustrate that 81.10 % of the productivity of clones SB04, SB11, SB19, and SB20 is influenced by genetic factors, while environmental factors influence 18.9 %.

The resistance to fire injury and coir content of superior clones SB04, SB11, SB19, and SB20 are presented in Table 8. As indicated in Table 8, the four clones demonstrated resistance to smut disease, as observed in both plant cane and ratoon cane until natural harvest. Moreover, they were also tested with a smut disease inoculant on cane plants up to the age of 6 months on polybag media, with only indicators of attack appearing at 6 months of age. Another supporting trait was that these four superior clones have the potential to produce high coir content in both plant cane and ratoon cane harvest methods, making them a potential source of bioenergy. The findings of this study corroborate that these four clones meet the requirements as candidates for new superior varieties, which aligns with previous studies (Kristini et al., 2022; Uchtiawati et al., 2020).

CONCLUSIONS

The genetic diversity among the four clones of *Saccharum officinarum* Linn is characterized by differences in morphological and agronomic traits, with their genetic superiority traits varying upon crossing, such as weight, yield, and crystallization. These clones exhibit high crystallization productivity exceeding 8-15 tons/ha due to superior genetic and morphological traits that cause differences in productivity. The superior clones of SB04, SB11, SB19, and SB20 resulted in higher sugarcane weight, yield, and crystal content than the two parents. SB04 yielded 13.41 tons/ha of crystals, SB11 produced 8.47 tons/ha of crystals, SB19 yielded 15.26 tons/ha of crystals, and SB20 produced 12.53 tons/ha of crystals, which were higher than those of their parent plants. The test results revealed a significant difference related to the dominance of the potential productivity variable; there were dominant clones in crystal production and some inherited traits from both parents that were high but not dominant.

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AUTHORS CONTRIBUTIONS

SB designed and conceived the experiments. WNL and AEP conducted the experiment. SB, WNL, and AEP contributed to sample preparation and interpretation of results. WNL and GSS primarily prepared this manuscript. All authors provided critical feedback and contributed to the research development, analysis, and manuscript.

COMPETING OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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