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BREEDING OF LOCAL GARLIC *(Allium sativum L.)* VARIETIES USING THE CHEMICAL MUTAGEN SODIUM AZIDE

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ABSTRACT

The demand for garlic in Indonesia continues to rise annually; however, the market remains dominated by imported garlic due to its larger bulb size compared to local varieties. One approach to improving the quality of local garlic is through mutation breeding using chemical mutagens such as sodium azide. This study aimed to evaluate the effects of sodium azide on morphological traits, stomatal index, and chlorophyll a and b content in local garlic (Allium sativum L.). The experiment was conducted using a Randomized Block Design (RBD) with a single treatment factor, sodium azide concentrations of 0.1%, 0.2%, 0.3%, and 0.4%. Observations revealed that the 0.4% concentration produced the best performance in plant height (24.20 cm), number of leaves (3.40), leaf length (22.10 cm), and leaf width (2.60 cm). The highest stomatal index (0.35) was also observed at this concentration. In contrast, the longest root length (2.320 cm) and highest fresh weight (0.8040 g) were found at the 0.3% concentration. The highest chlorophyll a and b content was recorded at the 0.1% concentration. These findings indicate that sodium azide induces significant variation in plant traits. Therefore, chemical mutagenesis is recommended as a promising breeding strategy to develop superior and competitive local garlic varieties.

Keywords: *Mutation Breeding, Sodium Azide, Allium Sativum, Morphological Traits*

INTRODUCTION

Indonesia is known as a country with an agrarian-based economy, where the majority of the population still relies on the agricultural sector and natural resource management for their livelihood (Sujana, 2020). Within this sector, agriculture not only supports the basic needs of the population by providing food, but also serves as a pillar of economic resilience, especially in the face of global fluctuations. As the population continues to grow each year, the demand for food commodities has also increased significantly (Aryawati, 2018). This puts pressure on agricultural productivity, including horticultural commodities such as garlic. Garlic (*Allium sativum L.*) is one of the most widely consumed horticultural crops in Indonesia, commonly used as a flavoring and taste enhancer in various traditional and modern dishes (Fadila & Respatijarti, 2018). Unfortunately, national garlic production is still unable to meet domestic demand. Data from the Ministry of Agriculture of the Republic of Indonesia shows that garlic consumption has increased by approximately 1.38% annually during the 2020–2024 period, while domestic production can only supply less than 10% of total national consumption (Dihni, 2022). This imbalance has led to a high dependence on imports, particularly from China.

One of the factors contributing to the low productivity of local garlic is its uncompetitive morphological characteristics. Small clove size, relatively long harvesting period, and low yield per hectare discourage farmers from planting local varieties (Bani et al., 2022). On the other hand, local garlic has advantages in terms of taste and aroma, which are stronger and more distinctive compared to imported garlic, giving it potential competitiveness from an organoleptic quality perspective (Bani, 2022). To enhance the competitiveness of local varieties, innovations in plant breeding are needed, one of which is through mutation induction approaches. Mutation induction is a technique used to create new genetic diversity by altering the DNA structure of plants, either through physical agents such as radiation or chemical agents such as mutagens (Husain, 2022). One commonly used chemical mutagen is sodium azide (NaN_3), due to its effectiveness in inducing mutations without causing severe damage to plant chromosomes and tissues (Nurhidayah, 2017; Amin et al., 2006).

Sodium azide works by modifying nitrogenous bases in DNA, causing genetic changes that can lead to desirable phenotypic variations. These mutations can be observed in various aspects of plant growth, such as plant height, number of leaves, root length, photosynthesis efficiency, and chlorophyll content (Marcu et al., 2013; Afify et al., 2011). This effect is highly promising for improving the agronomic performance of vegetatively propagated crops like garlic, which typically have low genetic diversity (Mensah et al., 2005). This study was conducted to evaluate the effects of sodium azide application on various important growth parameters in local garlic, including morphology, stomatal index, and chlorophyll a and b content. The results of this study are expected to offer an alternative strategy for improving the quality of local seed stock through mutation breeding technology, while also contributing to the expansion of genetic diversity in garlic varieties in Indonesia.

RESEARCH METHODOLOGY

This study used a one-factor Randomized Complete Block Design (RCBD) to evaluate the effect of different concentrations of the chemical mutagen sodium azide (NaN_3) on local garlic (*Allium sativum L.*). The treatment factor consisted of four sodium azide concentrations: 0.1%, 0.2%, 0.3%, and 0.4%. Each concentration was replicated five times, resulting in a total of 20 experimental units. The research was conducted over six months, from January to June 2024. Cultivation and plant character observations were carried out in Baturiti Village, Tabanan Regency, while stomatal and chlorophyll analyses were conducted at the Basic Laboratory of Dhyana Pura University and the Integrated Service Laboratory of the Faculty of Agricultural Technology, Udayana University.

Preparation of Materials and Mutagen Solution

Local garlic cloves were first soaked in distilled water (aquadest) for 6 hours for initial hydration, followed by immersion in sodium azide solution at the designated concentrations for 4 hours. After soaking, the cloves were rinsed with clean water and then soaked again in distilled water for 30 minutes, followed by two rinses to remove any residual mutagen solution (Sari, 2015). The solution formulation followed the procedure of Fazilatul (2023), where sodium azide and ethanol were mixed into distilled water to obtain a homogeneous mutagen solution.

Nursery and Maintenance

The treated garlic cloves were sown in 20x10 cm polybags filled with sterilized soil media. During the growth period, watering was done twice a day (morning and evening), and liquid fertilizer along with botanical insecticides was applied weekly to support plant growth and protection (Fadila & Respatijarti, 2018).

Observation of Plant Morphology

Morphological parameters observed included time of shoot emergence, plant height, number of leaves, leaf length and width, number and length of roots, fresh bulb weight, as well as visual descriptions of bulbs and leaves. Measurements were taken in the 11th week after planting. Leaf and bulb colors were identified using the RHS Colour Chart as the standard color reference (Friska, 2017).

Stomatal Observation

The stomatal index was calculated using the stomatal impression method, in which the lower leaf surface (abaxial) was coated with a clear acetone-based nail polish. Once dried, clear adhesive tape was used to lift the epidermal imprint. The imprint was mounted on a microscope slide and observed under a light microscope (Fauziah, 2019). The stomatal index was calculated according to the formula by Pharmawati (2015):

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$$\text{Stomatal Index: } \frac{S}{(S+E)}$$

Where:

- I = Stomatal index
- S = Number of stomata
- E = Number of epidermal cells

Chlorophyll Content Analysis

The analysis of chlorophyll a and b levels was conducted using an extraction method with 96% alcohol. A total of 0.1 grams of leaf tissue (excluding leaf veins) was crushed using a mortar, mixed with alcohol, and filtered. The filtrate was placed into a cuvette, and its absorbance was measured using a UV Spectrophotometer to determine the chlorophyll concentration (Amrullah, 2019).

RESULTS AND DISCUSSION

Morphological Characteristics

Observations of early sprout growth in garlic showed that treatment with sodium azide concentrations of 0.4% and 0.3% induced earlier shoot emergence, occurring on the 4th day after planting. In contrast, shoots in the control plants (without mutagen treatment) appeared later, as shown in Figure 1. This phenomenon indicates that chemical mutagen application can accelerate the shoot initiation process. According to Mutmainnah (2016), this accelerated growth is suspected to be related to the influence of mutagens on the activity of the plant's endogenous hormones. Sodium azide may trigger physiological changes that affect the balance of hormones such as auxin and cytokinin, which play a key role in stimulating cell division and shoot growth. Variations in plant response may also be due to natural differences in endogenous hormone content among individual plants, causing the response to mutagen treatment to vary depending on each explant's sensitivity level to sodium azide.

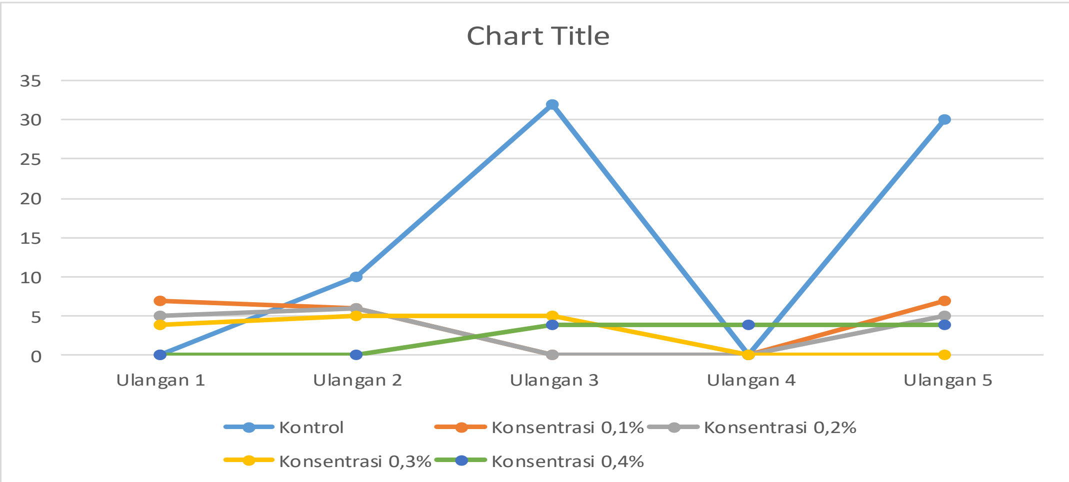

Figure 1. Days of Shoot Emergence

Based on the application of chemical mutagens, it was proven that the early growth process of garlic plants was influenced, as indicated by the fastest growth rate in the highest concentration treatment (0.4%). This treatment accelerated shoot initiation compared to lower concentrations. This is supported by Randi (2015), who stated that without mutagen intervention, plant growth processes can significantly slow down or even fail to occur. This finding is consistent with the control treatment, where shoot emergence only began between days 10 and 32, which was much slower compared to the sodium azide treatments. The absence of a mutagen in the control group hindered the physiological activity needed for shoot initiation, resulting in suboptimal growth compared to the sodium azide-treated plants. A similar study by Endang (2017) on *M1* generation rice (variety "kulit manis") showed that a sodium azide concentration of 4.0 mM resulted in the highest germination rate (81.50%) during early observations, whereas 2.0 mM produced only 60.00%. This indicates that the effectiveness of a mutagen depends on the dosage used and that certain concentrations can significantly accelerate germination. However, excessively high doses may inhibit the biological and physiological activity of seeds, including enzyme activity, hormonal imbalances, and mitotic disturbances caused by disruptions in the cellular respiration chain. Sodium azide contains azide anions, which are strong inhibitors of cytochrome oxidase enzymes, disrupting oxidative phosphorylation and inhibiting energy metabolism (Endang, 2017).

Differences in shoot emergence timing and plant mortality rates are likely influenced by the physiological capacity of cells in the explant tissue following mutagenic treatment. Internal factors such as genetic variation and the sensitivity of meristematic cells to mutation largely determine how explants respond to treatment. Each meristem cell has regenerative potential to form shoots, but mutagen effects may cause uneven physiological changes depending on the stability and resilience of the cells under chemical stress. This is explained by Qosim (2019) as an individual genotypic response that determines the success of shoot regeneration. In addition to genetic factors, environmental influences (external factors) also play a role in vegetative plant growth. Variables such as sunlight intensity, water availability, nutrients, temperature, and humidity affect key physiological processes like photosynthesis and transpiration. According to Vinanda (2020), light is one of the dominant factors in stimulating vegetative growth, as it directly affects photosynthetic efficiency, which ultimately influences shoot growth rate and overall plant development.

The Mutagenic Effect of Sodium Azide on Garlic Growth

Sodium azide (NaN_3) is one of the effective chemical mutagens used to increase plant genetic diversity through mutation induction. The use of NaN_3 in garlic has shown significant effects on various growth parameters. Research by Mahajan et al. (2015) demonstrated that treatment of garlic variety G-41 with 0.01% NaN_3 for 12 hours resulted in optimal plant growth compared to higher concentrations. At concentrations of 0.04% and 0.08%, there was a decline in plant height and leaf number, as well as increased plant mortality. Concentrations of 0.10% and above even caused total plant death. Another study by Türkoğlu et al. (2022) on wheat indicated that NaN_3 can cause genomic instability and polymorphisms, showing high mutagenic potential. However, these effects are highly dependent on the concentration and duration of treatment. In the context of garlic, using NaN_3 as a mutagen can accelerate shoot initiation and increase genetic variation, which is crucial for plant breeding programs. However, it is essential to determine the appropriate dosage to avoid harmful toxic effects. The use of sodium azide as a chemical mutagen in garlic can enhance genetic diversity and accelerate shoot growth, but its effectiveness depends heavily on treatment concentration and duration. Excessively high concentrations can cause toxicity and plant death. Therefore, determining the correct dosage is critical in the application of mutagenesis for garlic plant breeding.

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Table 1. Morphological Analysis Results of Garlic Plants

Treatment Concentration	Plant Height	Number of Leaves	Leaf Length	Leaf Width	Number of Roots	Root Length
Control	14,34[a]	2,00[a]	12,60[a]	0,94[a]	**11,60[a]**	1,920[a]
0,1%	21,70[a]	2,60[a]	19,94[a]	1,64[a]	5,40[a]	1,180[a]
0,2%	22,28[a]	2,80[a]	20,28[a]	1,90[a]	10,80[a]	1,560[a]
0,3%	22,40[a]	3,20[a]	20,40[a]	2,32 [a]	**11,60[a]**	**2,320[a]**
0,4%	**24,20[a]**	**3,40[a]**	**22,10[a]**	**2,60[a]**	**11,60[a]**	2,220[a]

Note: Different letter notations in the same column indicate significant differences ($P < 0.05$).

The morphological analysis results of local garlic presented in Table 1 show varying plant responses to the application of sodium azide mutagen at different concentrations. In general, morphological parameters of the upper plant parts, such as plant height, number of leaves, leaf length, and leaf width experienced significant increases under sodium azide treatments, with the 0.4% concentration yielding the most optimal results (Nurhidayah, 2017; Sari, 2015; Fajriyah, 2019). This increase is assumed to result from the activity of sodium azide as a mutagenic agent stimulating the production of plant growth hormones, especially auxin. This hormone is known to play a key role in cell division, tissue elongation, and vegetative organ formation (Sari, 2022; Viana, 2019). Similar findings were reported by Nurhidayah (2017) in rice, and by Sari (2015, 2022) in chili and tomato plants, showing that chemical mutation treatments can accelerate growth and increase the size of vegetative organs compared to control plants.

Sodium azide has been proven to enhance plant genetic diversity through the induction of positive mutations, thereby accelerating the selection of superior traits in the plant breeding process (Fajriyah, 2019; Viana, 2019). However, a negative response was detected in root system parameters. Although the number of roots did not differ significantly from the control, root length tended to decrease in the 0.1%, 0.2%, and 0.4% treatments, indicating signs of physiological stress due to sodium azide toxicity (Mshembula, 2017). This phenomenon is likely related to disturbances in enzymatic activity and inhibition of cell mitosis in root tissues, leading to slower development of the plant's lower organs (Endang, 2017). Therefore, the use of sodium azide as a mutagen must be applied carefully with optimal dose adjustments to ensure that the improvement of above-ground plant parts does not come at the expense of root health or the overall root system (Sari, 2022).

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Table 2. Average Fresh Weight of Garlic Plants

Treatment Concentration	Average Fresh Weight Results
Control	0,6060[a]
0,1%	0,4300[a]
0,2%	0,3320[a]
0,3%	**0,8040[a]**
0,4%	0,6680[a]

Note: Different letter notations in the same column indicate significant differences (P < 0.05).

The analysis of fresh weight in local garlic plants showed that sodium azide treatment at concentrations ranging from 0.1% to 0.4% did not result in statistically significant differences compared to the control. Interestingly, the highest average value was observed at the 0.3% concentration (0.8040 grams), but further analysis using Duncan's test at a 5% significance level indicated that the difference was not statistically meaningful (Husain, 2021). Although sodium azide demonstrated significant effects on morphological aspects such as plant height, leaf length, and number of leaves, the fresh weight parameter did not follow the same trend. This indicates that phenotypic transformation in vegetative organs is not necessarily accompanied by an increase in fresh biomass (Sari et al., 2015). The decrease or non-significant changes in fresh biomass are most likely related to the physiological effects of the mutagen that disrupt the plant's metabolism (Kharde, 2017). Sodium azide is known to act as a mutagen by inducing point mutations in DNA, but not all resulting genetic changes are beneficial from an agronomic perspective (Girija, 2019). Random mutations can disrupt the expression of genes responsible for cellular respiration and energy metabolism, such as ATP synthesis, which directly impacts biomass accumulation (Kharde, 2017; Girija, 2019). A reduction in fresh weight may also result from impaired photosynthesis and respiration, which are essential for the formation of new tissues (Nurhidayah, 2017).

Nurhidayah (2017) reported that at high concentrations, sodium azide is toxic because it can inhibit mitochondrial enzymes such as cytochrome oxidase. This enzyme is crucial for oxidative phosphorylation, and when inhibited, the plant's ability to generate energy through aerobic respiration becomes compromised. As a result, biomass growth is suboptimal, even if other growth parameters show improvement. Furthermore, visual observations using the RHS Colour Chart (2017) indicated phenotypic variations in leaf color and bulb shape across different treatments. These changes may be due to disruptions in the expression of genes regulating pigment biosynthesis, such as chlorophyll, anthocyanins, or carotenoids, which are often indicators of physiological stress from mutagen exposure (Girija, 2019). Darker leaf colors or changes in surface glossiness in plants treated with mutagens also suggest a decrease in photosynthetic efficiency and plant adaptation to stress (Kharde, 2017).

Table 3. Plant Characterization Results

Treatment Concentration	Bulb Shape	**Bulb Color**	Leaf Color
Control	Oval	156C (*Linen*)	140A (*Forest green*)
0,1%	Oval	155D (*Antique white*)	134A (*Dark green*)
0,2%	Oval	155D (*Antique white*)	140A (*Forest green*)
0,3%	Round	156D (*Linen*)	140A (*Forest green*)
0,4%	Oval	NN155C (*Beige*)	140A (*Forest green*)

Note: Different letter notations in the same column indicate significant differences ($P < 0.05$).

Morphological Characterization and Stomatal Index of Garlic Plants

The morphological characterization of garlic plants treated with sodium azide showed variations in bulb shape and color as well as leaf color, indicating mutagenic effects on phenotypic gene expression. The 0.3% concentration treatment resulted in round bulb shapes and consistently dark green leaf color (RHS code 140A), differing from the control and other treatments, which mostly produced oval bulbs (RHS, 2017). This change in shape suggests a possible mutation in genes regulating cell division in the bulb tissues (Girija, 2019). Changes in bulb color, such as turning beige (NN155C) or antique white (155D), indicate potential alterations in pigment and phenolic compound synthesis, which may also reflect adaptations to mutagenic stress (Kharde, 2017). Furthermore, the stomatal index observations indicated that sodium azide at a concentration of 0.3% produced the highest stomatal index value. This suggests that at an optimal dose, the mutagen can increase stomatal density, contributing to the plant's physiological efficiency in CO_2 uptake and photosynthesis (Taiz & Zeiger, 2010; Pharmawati, 2015). However, increasing the mutagen concentration to 0.4% resulted in a decrease in the index, possibly due to toxic effects that reduced epidermal cell division and stomatal size (Setyowati, 2015).

Setyowati (2015) emphasized that mutations can enlarge stomatal size, but this often leads to a reduced stomatal density per unit leaf area, thereby decreasing the stomatal index. This phenomenon is frequently observed in plants that have undergone polyploid mutation. In the context of this study, variations in the stomatal index also correlated with the time of observation. Anni et al. (2018) showed that observations made in the morning, as light intensity begins to rise, will show more stomata in an open state. This stomatal response to light allows increased photosynthesis when plants are in optimal conditions. Cambaba et al. (2019, 2022) added that stomatal density and activity are strongly influenced by environmental factors such as temperature and humidity. High stomatal density can accelerate transpiration rates and CO_2 uptake; however, if not accompanied by efficient water regulation, plants may experience water stress. Therefore, mutations that can regulate the stomatal index to an optimal level—not too high or too low—will be an added value in mutation-based breeding programs (Pharmawati, 2015).

Stomatal Index

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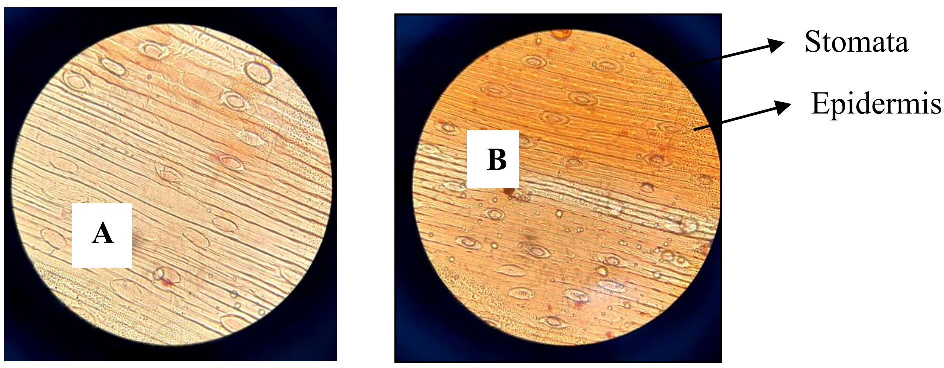
The analysis of the stomatal index showed that treatment with sodium azide at various concentrations did not result in statistically significant differences in the stomatal index values of garlic plants. However, the treatment with a 0.3% concentration showed the highest average stomatal index compared to the other treatments, as listed in Table 4. A higher stomatal index reflects an increase in stomatal density on the leaf surface, which physiologically may accelerate the plant's transpiration rate. Leaf sampling for stomatal observations was conducted in the morning between 09:00 and 10:00 WITA, a time when stomata tend to be open. This is due to the relatively low light intensity in the morning, which has not yet triggered the plant's active stomatal closure mechanisms.

Table 4. Average Stomatal Index Results

Treatment Concentration	Average Stomatal Index
Control	0,334[a]
0,1%	0,342[a]
0,2%	0,329[a]
0,3%	**0,349[a]**
0,4%	0,341[a]

Note: Different letter notations in the same column indicate significant differences (P < 0.05).



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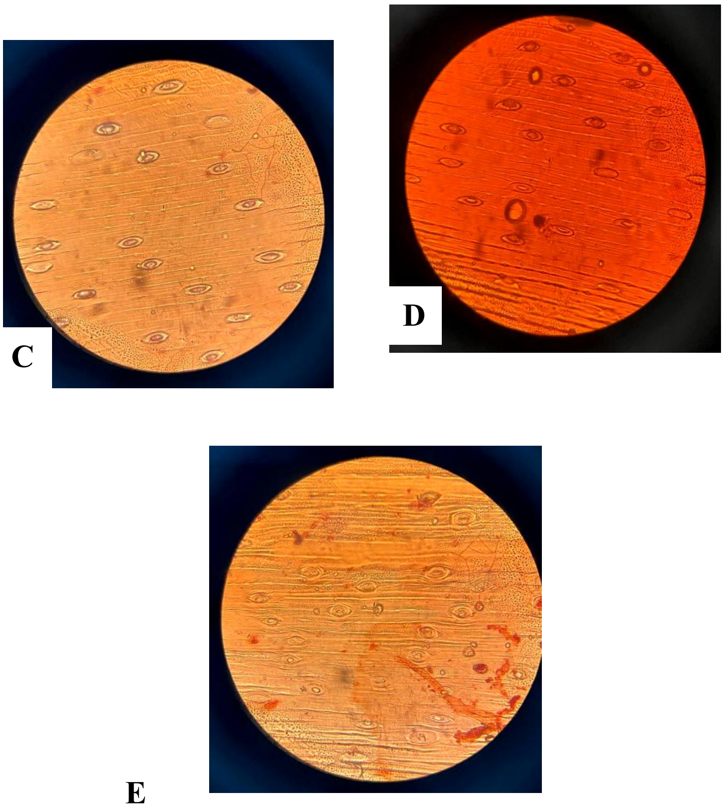

Figure 2. Stomatal Index Results.
Description: A. Control, B. 0.1% Concentration, C. 0.2% Concentration, D. 0.3% Concentration, E. 0.4% Concentration
Source: Personal Documentation

Observations of the stomatal index in garlic plants showed that sodium azide treatments at different concentrations did not produce a statistically significant effect on the average stomatal index (see Table 4). However, the treatment with a 0.3% concentration yielded the highest average value (0.349), while the lowest value was found at the 0.2% concentration (0.329), which was even lower than the control (0.334). This indicates a fluctuating physiological response of the plant to sodium azide mutagen, particularly in its effect on stomatal density (Pharmawati, 2015). Stomata are important epidermal structures in the form of pores controlled by two guard cells, functioning in the regulation of transpiration and photosynthesis (Izza et al., 2015; Taiz & Zeiger, 2010). Changes in the stomatal index can serve as an indirect indicator of ploidy level alterations or physiological conditions resulting from mutagenic treatment (Setyowati, 2015). A higher stomatal index means more stomata per unit leaf area, which can enhance transpiration and the efficiency of gas exchange (CO_2 and O_2) during photosynthesis.

Pharmawati (2015), in her study on garlic plants, showed that high concentrations of colchicine significantly reduced the stomatal index compared to the control. A similar phenomenon was observed in this study, where treatment with a high concentration of sodium azide (0.4%) produced a lower index than the moderate concentration (0.3%). This finding supports the hypothesis that optimal mutagen concentrations can improve or stimulate physiological traits, while excessive doses may be toxic. Setyowati (2015) emphasized an inverse relationship between stomatal size and density. Chemical-induced mutations can enlarge stomatal size, which in turn leads to a decrease in the index. Therefore, the higher average stomatal index at the 0.3% concentration may be associated with smaller stomatal size and higher density.

Stomatal observations were conducted in the morning between 09:00 and 10:00 WITA, when light intensity was still low and the stomata were generally open. This is consistent with the findings of Cambaba et al. (2019), who reported that in *Syzygium oleina* (red shoot) plants, the highest number of open stomata was recorded at 08:00 a.m., with a drastic decrease occurring at midday due to increased temperature and light intensity. Cambaba (2022) further explained that environmental factors such as temperature, light, and water availability greatly influence the daily rhythm of stomatal opening and closing. Based on these findings, it can be concluded that sodium azide mutagen concentration affects the stomatal index in a fluctuating manner, with 0.3% as the optimal point. However, the lack of a statistically significant effect suggests that external factors and the plant's physiological interactions also influence stomatal responses.

Chlorophyll A and B Analysis

Table 5. Results of Chlorophyll A and B Analysis

Treatment Concentration	Chlorophyll A (mg/L)	Chlorophyll B (mg/L)
Control	600,8818	328,646
0,1%	**839,8024**	**392,562**
0,2%	714,9737	325,880
0,3%	603,6393	277,375
0,4%	457,7534	228,135

Note: Different letter notations in the same column indicate significant differences (P < 0.05).

Chlorophyll Content Analysis Results

The analysis of chlorophyll content in garlic leaves showed that the application of sodium azide mutagen significantly affected chlorophyll A and B levels. Treatment with a 0.1% concentration resulted in the highest chlorophyll A content at 839.80 mg/L and chlorophyll B at 392.56 mg/L, indicating stimulation of photosynthetic pigment biosynthesis at low concentrations (Novitasari, 2023). Conversely, a 0.4% concentration showed a significant decline, with chlorophyll A levels at only 457.75 mg/L and chlorophyll B at 228.13 mg/L, indicating toxic effects of the mutagen at high concentrations (Dahot, 2017). Correlation analysis between the two types of chlorophyll showed a strong relationship, with a Pearson value of 0.939 (p = 0.018), suggesting that an increase in chlorophyll A is generally followed by an increase in chlorophyll B, and vice versa. This confirms a metabolic link between the two pigments in the plant photosynthetic system (Rahmawati, 2018).

According to Novitasari (2023), colchicine treatment in garlic at appropriate doses can increase chlorophyll levels depending on the variety and growth stage. Chemical mutations are known to modify gene expression involved in chlorophyll biosynthesis pathways, thereby enhancing pigment synthesis to a certain extent. However, these results are not universal. Dahot (2017) reported that sodium azide significantly reduced chlorophyll levels, especially in legumes, showing susceptibility to oxidative stress due to mutation. Meanwhile, Gnanamurthy (2015) stated that sodium azide was ineffective in increasing chlorophyll levels, although it had a positive effect on other metabolite contents such as protein and reducing sugars. The distribution of chlorophyll A and B in plant tissues is physiological and functionally distinct. Chlorophyll A is the primary pigment in photosynthesis and tends to dominate in older leaf tissues, while chlorophyll B acts as an accessory pigment that aids in light absorption at specific wavelengths and is more abundant in young leaves (Rahmawati, 2018). The decrease in chlorophyll content in high-concentration sodium azide treatment is likely triggered by disruptions in chlorophyll biosynthesis enzyme activity, oxidative stress, or adaptive mechanisms of the plant to environmental pressure (Amrullah, 2019). Additionally, chlorophyll content is strongly influenced by environmental factors such as light intensity, temperature, humidity, and water availability. Under environmental stress conditions like drought, plants tend to reduce chlorophyll content to lower energy and water consumption, which directly affects photosynthesis efficiency and growth (Amrullah, 2019).

CONCLUSIONS AND RECOMMENDATIONS

Based on the research findings, it can be concluded that the application of sodium azide mutagen at concentrations of 0.1%, 0.2%, 0.3%, and 0.4% had an effect on the morphological and physiological growth characteristics of garlic plants, although not all parameters showed significant differences compared to the control. The best morphological treatment was observed at the 0.4% concentration, which resulted in the highest plant height, number of leaves, leaf length, and width, while the longest root and highest fresh weight were achieved at the 0.3% concentration. On the other hand, the stomatal index did not show significant differences among treatments, with the highest value at 0.4% and the lowest at 0.2%. The analysis of chlorophyll a and b content showed that the 0.1% concentration resulted in the highest chlorophyll levels, while the 0.4% concentration significantly decreased chlorophyll content. These findings indicate that the use of chemical mutagens like sodium azide can selectively influence garlic plant growth depending on the concentration used. The novelty of this study lies in its application to vegetative crops such as garlic, which have been relatively understudied in the context of chemical mutagenesis. It also provides preliminary information on the potential of sodium azide to enhance genetic variability and initial morphological traits for plant breeding purposes. To obtain more significant and applicable results, it is recommended that future research explore sodium azide concentrations higher than 0.4%, while still considering the safety threshold for plants. Furthermore, observation of the next generation is strongly advised to evaluate the stability of mutated traits, along with molecular testing and other agronomic parameters such as the number and size of bulbs, to support the development of superior garlic varieties more comprehensively.

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