

Research Article

Morphological and physiological responses of ornamental banana (*Musa ornata* Roxb.) to sodium azide mutagenesis

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Abstract - Induced mutagenesis represents a practical approach for generating genetic variability in ornamental bananas, where conventional breeding is constrained by sterility and parthenocarpy. This study evaluated the morphological and physiological responses of *Musa ornata* Roxb. plantlets to sodium azide (NaN_3) treatment under *in vitro* conditions. Uniform plantlets were exposed to NaN_3 concentrations ranging from 0.0 to 4.0 mM for 1 h, and survival rate, growth-related traits, photosynthetic pigment contents, and leaf color parameters were assessed after 16 weeks of culture. Survival rate declined in a clear concentration-dependent manner, allowing the estimation of LD_{30} and LD_{50} values at 1.13 and 2.08 mM, respectively. Among the evaluated traits, fresh weight showed a statistically significant increase at a low NaN_3 concentration (0.1 mM), whereas leaf number, pseudostem length, root traits, photosynthetic pigments, and leaf color parameters exhibited only numerical variation without consistent statistical significance. Chlorophyll and carotenoid contents, as well as CIELAB leaf color parameters, did not differ significantly among treatments. These results indicate that NaN_3 primarily affects *M. ornata* plantlets through concentration-dependent survival responses, while most morphological and physiological traits display descriptive variation at the *in vitro* stage. The study provides species-specific lethal dose reference values and baseline phenotypic screening information for the application of NaN_3 in ornamental banana mutation breeding. Further studies are required to determine the stability and breeding value of NaN_3 -induced variations after the early screening stage.

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1. Introduction

Ornamental bananas (*Musa* spp.) are widely cultivated in tropical and subtropical regions for their striking foliage and colorful inflorescences, making them valuable components of the landscaping and floriculture industries (Kew Science, n.d.; Gardenia.net, n.d.). Beyond their ornamental appeal, bananas play an important role in the horticultural sector by generating economic benefits for growers and supporting local and regional markets, as highlighted by the Food and Agriculture Organization of the United Nations (n.d.). In addition, bananas are widely recognized as a nutritionally important crop, providing essential carbohydrates, dietary fiber, vitamins such as vitamin C and B6, and minerals including potassium and magnesium, which contribute to human nutrition and health (Harvard T.H. Chan School of Public Health, n.d.). Among ornamental bananas, *Musa ornata* Roxb., commonly known as the pink-flowered banana, has gained increasing popularity as an ornamental crop due to its vibrant bracts and adaptability to various cultivation systems. However, the genetic improvement of ornamental bananas faces formidable challenges that severely constrain conventional breeding approaches. Sterility, low seed germination rates, and parthenocarpic fruit development restrict the availability of natural genetic variation in these species (Kumar, 2006; Waniale et al., 2024). These biological limitations create a need for alternative breeding strategies to broaden the genetic base and enhance the aesthetic and commercial value of ornamental bananas. Induced mutagenesis has emerged as a powerful and practical strategy for generating genetic variability in plant species where sexual reproduction is constrained or inefficient. Recent advances in banana molecular breeding have highlighted mutation breeding as a cornerstone technology for trait improvement,

particularly in cultivars where traditional hybridization is not feasible (Cheng et al., 2024). Further studies are required to determine the stability and breeding value of NaN_3 induced variations after the early screening stage that may translate into phenotypic variation with potential horticultural value (Saraswathi et al., 2020; Robertson, 2024). Among chemical mutagens, NaN_3 is particularly attractive due to its relatively low cost, high mutation efficiency, and reduced handling risk compared with EMS (Galatalı, 2023). The mutagenic mechanism of NaN_3 involves the generation of reactive nitrogen species and direct interaction with DNA, leading to base-pair substitutions, particularly G:C to A:T transitions (Veleminský & Angelis, 1987).

The effectiveness of NaN_3 in generating heritable mutations has been extensively demonstrated across a wide range of crop species, including cereals, legumes, and ornamentals (Adamu & Aliyu, 2007; Srivastava et al., 2011; Türkoğlu et al., 2023). Recent breakthroughs in ornamental plant improvement have further validated the utility of sodium azide mutagenesis under *in vitro* conditions. For instance, Wu et al. (2024) successfully applied NaN_3 to induce mutations and promote early flowering in *Cymbidium faberi* orchids, demonstrating the feasibility of this approach for enhancing ornamental traits in tissue culture systems. Similarly, advances in mutation breeding across horticultural crops have underscored the importance of optimizing mutagen dosage and treatment protocols to balance mutation induction with plant viability (Galatalı, 2023; Robertson, 2024). Despite these advances, the application of NaN_3 in ornamental bananas, particularly *M. ornata*, remains largely unexplored, leaving a significant gap in both scientific knowledge and practical breeding strategies for this commercially valuable species.

Plant tissue culture provides a reliable and reproducible system for producing uniform explants suitable for mutagen treatment and for regenerating mutant lines under controlled environmental conditions. The integration of tissue culture with chemical mutagenesis not only ensures experimental reproducibility but also enhances the likelihood of recovering viable mutants with desirable ornamental traits (Wannajindaporn et al., 2016; Hernández-Muñoz et al., 2019). Furthermore, the systematic evaluation of morphological and physiological responses—including survival rates, growth performance, leaf and root development, and photosynthetic pigment content—serves as an essential preliminary step for determining lethal dose thresholds (LD_{30} , LD_{50}) and for identifying promising phenotypic variants prior to molecular characterization.

While previous studies have investigated sodium azide mutagenesis in various plant species (Ganesan et al., 2005; Adamu & Aliyu, 2007; Srivastava et al., 2011), research specifically targeting ornamental bananas under tissue culture conditions is notably scarce. The present study addresses this gap by providing the first comprehensive assessment of NaN_3 -induced morphological and physiological responses in *M. ornata* plantlets. Unlike earlier mutagenesis studies in bananas that focused primarily on edible cultivars for agronomic trait improvement (Saraswathi et al., 2020; Robertson, 2024), this research emphasizes ornamental characteristics and integrates detailed pigment analysis with growth parameter assessments to identify phenotypic variations with ornamental value. Moreover, this study establishes species-specific lethal dose parameters and hormetic response thresholds that are critical for designing future mutation breeding programs in ornamental banana improvement.

The present study aimed to investigate the morphological and physiological

effects of NaN_3 -induced mutagenesis in *M. ornata* plantlets derived from tissue culture. Specifically, the objectives were (i) to determine the impact of different NaN_3 concentrations on plant survival and growth characteristics, (ii) to estimate LD_{30} and LD_{50} values as reference points for mutagen application, and (iii) to assess phenotypic alterations in vegetative traits and photosynthetic pigment composition. The outcomes of this research are expected to provide an evidence-based foundation for optimizing mutagen treatments in ornamental bananas and to facilitate the development of novel cultivars with enhanced ornamental traits and commercial potential.

2. Materials and methods

2.1 Plant material and tissue culture establishment

Plantlets of *M. ornata* Roxb. were obtained from healthy suckers collected from commercial plantations in northeastern Thailand. Explants were surface-sterilized with 70 % ethanol for 1 min, followed by immersion in 20 % (v/v) commercial sodium hypochlorite containing one drop of Tween-20 for 15 min, and then rinsed three times with sterile distilled water. Sterile shoot tips were cultured on Murashige and Skoog (MS) basal medium supplemented with 5.0 mg L^{-1} 6-benzyladenine (BA) to induce multiple-shoot formation. Subcultures for multiplication were maintained on MS medium containing 2.0 mg L^{-1} BA until sufficient uniform plantlets were obtained for mutagen treatment.

2.2 Mutagen treatment

Uniform *in vitro* grown plantlets (2–3 cm tall) were immersed in sodium azide (NaN_3) solutions at concentrations of 0.0, 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 mM prepared in 0.1 M citrate–phosphate buffer at pH 5.0 ± 0.1 . The pH was carefully adjusted and monitored before and after treatment to minimize NaN_3 decomposition, as the

compound rapidly releases toxic hydrazoic acid under acidic conditions (Raina et al., 2021). The buffer consisted of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate mixed in a 1:1 ratio to achieve pH 5.0, ensuring moderate acidity while maintaining NaN_3 stability during treatment.

Exposure duration was fixed at 1 h based on preliminary screening (data not shown) and supported by earlier mutagenesis studies reporting that 1 h provides effective mutagenic action with acceptable survival in *in vitro* systems (Wannajindaporn et al., 2016; Wu et al., 2024). After treatment, plantlets were rinsed three times with sterile distilled water to remove residual NaN_3 and transferred to hormone-free MS medium for recovery and subsequent growth.

2.3 Experimental design and statistical analysis

The experiment followed a Completely Randomized Design (CRD) with three replicates per treatment and ten plantlets per replicate. Randomization of plantlets among treatment trays was performed using a computer-generated list to minimize positional bias. Data for all quantitative variables (survival rate, growth, and pigment traits) were subjected to statistical analysis to compare the effects of different NaN_3 concentrations among treatments. Prior to analysis,

datasets were tested for compliance with the assumptions of analysis of variance (ANOVA). Normality of residuals was evaluated using the Shapiro–Wilk test, and homogeneity of variances (homoscedasticity) was verified using Levene’s test (Field, 2018). When assumptions were violated, data were log- or square-root-transformed to improve normality and variance equality. Statistical analyses were performed using IBM SPSS Statistics v.29 (IBM Corp., Armonk, NY, USA). Treatment effects were analyzed by one-way ANOVA, and comparisons among NaN_3 concentration levels were conducted using Duncan’s Multiple Range Test (DMRT) at $p < 0.05$ to identify statistically significant differences between means. To improve interpretability, effect sizes (η^2) were calculated for all ANOVA models to indicate the proportion of total variance explained by NaN_3 concentration (Cohen, 1988).

Results are presented as mean $\pm$ standard deviation (SD) to facilitate comparison of variability among treatments. Regression analysis of the relationship between NaN_3 concentration and survival rate was performed to estimate lethal-dose values (LD_{30} and LD_{50}), with 95 % confidence intervals calculated for slope estimates.

Table 1. Experimental design and variables measured in *Musa ornata* plantlets treated with sodium azide (NaN₃).

Treatment (NaN ₃ concentration, mM)	Exposure time (h)	Replicates (n)	Plantlets per replicate	Total plantlets	Variables measured
0.0 (control)	1	3	10	30	Survival rate (%),
0.1	1	3	10	30	Fresh weight (g),
0.5	1	3	10	30	Number of leaves,
1.0	1	3	10	30	Leaf length (cm),
2.0	1	3	10	30	Leaf area (cm ²), pseu-
3.0	1	3	10	30	dostem length (cm),
4.0	1	3	10	30	Root number,
					Root length (cm),
					Leaf color parameters,
					Chlorophyll a,
					Chlorophyll b,
					Total chlorophyll,
					Carotenoids

Note: Plantlets were cultured under *in vitro* conditions and exposed to different NaN₃ concentrations for a fixed duration. Morphological and physiological variables were recorded after 16 weeks of culture.

2.4 Morphological and physiological assessments

After 16 weeks of culture, plantlets were evaluated for morphological and physiological parameters, including fresh weight (g plant⁻¹), number of leaves, leaf area (cm²), pseudostem length (cm), root number, and root length (cm). Leaf CIELAB color parameters (L*, a*, b*) were recorded using a Linshang LS173 Colorimeter (Shenzhen Linshang Technology Co., Ltd., China).

For pigment quantification, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were extracted from 0.1 g of fresh leaf tissue using 80 % acetone and measured spectrophotometrically at 663, 645, and 480 nm (Arnon, 1949; Lichtenthaler & Wellburn, 1983). Calculations were made using the standard equations:

$$\text{Chl a (mg g}^{-1} \text{ FW)} = (12.7 \times A_{663}) - (2.69 \times A_{645})$$

$$\text{Chl b (mg g}^{-1} \text{ FW)} = (22.9 \times A_{645}) - (4.68 \times A_{663})$$

$$\text{Total Chl} = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

$$\text{Carotenoids (mg g}^{-1} \text{ FW)} = (A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645}) \times V / (1000 \times W)$$

where A is absorbance, V is the extraction

volume (mL), and W is the sample fresh weight (g). All pigment analyses were performed in triplicate, and results were expressed as mean ± SE.

2.5 Ethical considerations

No human or animal subjects were involved in this study. All experiments complied with institutional biosafety regulations and followed the laboratory safety guidelines of Kalasin University.

3. Results and discussion

3.1 Effect of sodium azide on survival rate and determination of LD₃₀/LD₅₀

The survival percentage of *M. ornata* plantlets decreased markedly with increasing NaN₃ concentrations (Table 2). Control plants (0 mM) exhibited 100 % survival, whereas survival dropped to 88.89 %, 77.78 %, 66.67 %, 55.56 %, and 44.44 % at 0.1, 0.5, 1.0, 2.0, and 3.0 mM, respectively, with complete mortality at 4.0 mM. Regression analysis ($y = 20.92x + 6.41$, $R^2 = 0.94$) revealed LD₃₀ = 1.13 mM and LD₅₀ = 2.08 mM (Figure 1), both of which are indicated as dashed reference lines on the survival curve to enhance interpretability.

The concentration-dependent cytotoxicity aligns with findings in *Dendrobium* orchids and wheat, where NaN_3 disrupts cellular metabolism and survival beyond critical thresholds (Wannajindaporn et al., 2016; Türkoğlu et al., 2023). Estimation of LD_{30} and LD_{50} provides a quantitative foundation for balancing mutation induction and survival, serving as critical dose benchmarks for future mutagenesis studies (Adamu & Aliyu, 2007; Srivastava et al., 2011).

3.2 Morphological responses of *M. ornata* to NaN_3

3.2.1 Fresh weight and leaf number

Fresh weight increased significantly at 0.1 mM NaN_3 (16.94 g) compared with the control (15.68 g; $p < 0.05$), indicating a stimulatory effect at low mutagen concentration. In contrast, although leaf number showed a numerical increase at

0.1 mM (4.61 leaves plant⁻¹), this difference was not statistically significant compared with the control and other treatments (Table 3). Overall, growth responses across NaN_3 concentrations did not follow a strictly monotonic or consistent trend. Therefore, the observed increase in fresh weight at low NaN_3 concentration suggests a potential hormetic-like response, which should be interpreted with caution. Because no comparative figures illustrating morphological differences among individual treatments were included, the interpretation is based solely on quantitative statistical comparisons rather than visual assessment. Similar low-dose stimulatory effects have been reported in other plant species exposed to mild chemical stress; however, the magnitude and consistency of such responses are highly species- and trait-dependent (Wu et al., 2024; Lozada et al., 2025).

Table 2. Survival percentage of *Musa ornata* plantlets under different sodium azide (NaN_3) concentrations.

Treatment (mM)	Survival (%)
0 (control)	100.00 ± 0.00 ^a
0.1	88.89 ± 19.24 ^{ab}
0.5	77.78 ± 19.24 ^{abc}
1.0	66.67 ± 0.00 ^{bcd}
2.0	55.56 ± 19.25 ^{cd}
3.0	44.44 ± 19.25 ^d
4.0	0.00 ± 0.00 ^e

F-test (ANOVA): $F = 15.666$, $p < 0.001$

Coefficient of variation (CV): 54.61%

Note: Values are presented as mean ± standard deviation (SD). Different lowercase letters within the column indicate statistically significant differences among treatments according to Duncan’s Multiple Range Test (DMRT) at $p < 0.05$.

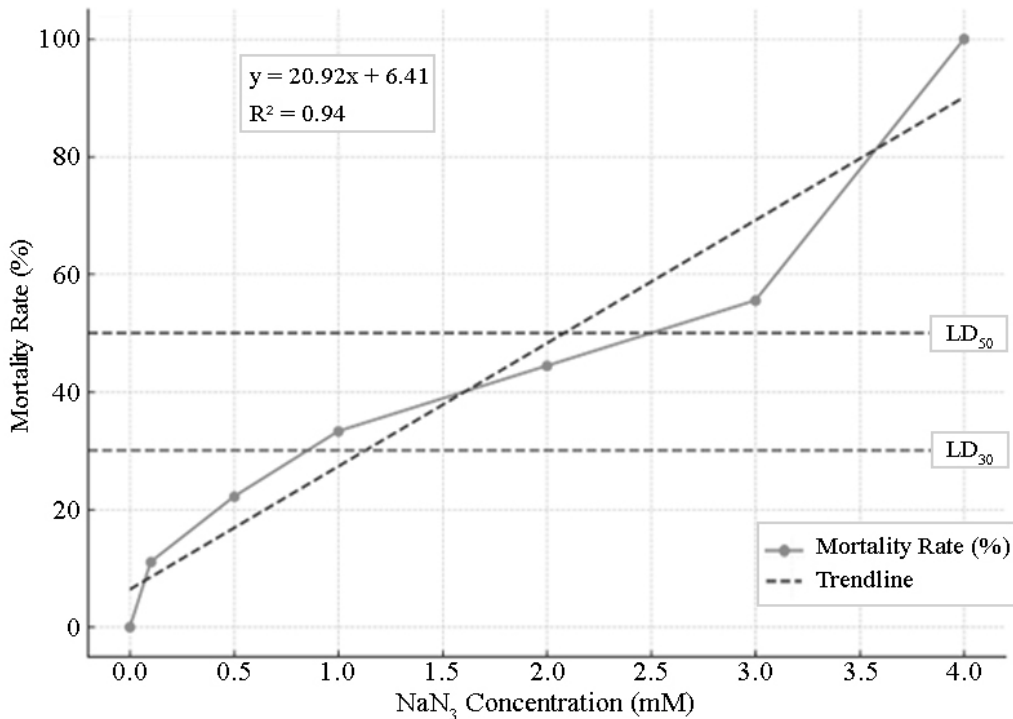


Figure 1. Mortality rate of *Musa ornata* plantlets as affected by sodium azide (NaN_3) concentration. The fitted regression line describes the relationship between NaN_3 concentration and mortality percentage ($R^2 = 0.94$).

3.2.2 Pseudostem and root traits

Root plasticity has been reported under various abiotic stress conditions; however, in the present study, pseudostem and root traits of *M. ornata* plantlets showed only numerical variation across NaN_3 concentrations without statistical significance. Therefore, the observed differences should be interpreted descriptively, and no adaptive or compensatory root growth response can be conclusively inferred from the current data.

In rice, stress-induced expression of *OsWnk9* has been shown to promote root elongation and stomatal regulation via hormonal signaling networks, suggesting that moderate abiotic stress may activate growth-related pathways rather than exclusively inhibitory responses (Negi &

Kumar, 2025). In the present study, root length of *M. ornata* plantlets exhibited numerical variation across NaN_3 concentrations; however, these differences were not statistically significant. Therefore, while the observed trends are qualitatively consistent with stress-related root plasticity reported in previous studies, the current data do not provide sufficient statistical evidence to confirm that NaN_3 induced a comparable adaptive or compensatory root growth response in *M. ornata*. Future investigations integrating physiological and molecular analyses, such as hormonal balance, antioxidant enzyme activities, and ROS dynamics, are required to determine whether NaN_3 exposure can elicit stress-mediated growth responses in *Musa* beyond random phenotypic variation.

Table 3. Effects of sodium azide (NaN_3) on morphological traits of *Musa ornata* plantlets after 16 weeks of *in vitro* culture.

NaN_3 (mM)	Fresh weight (g)	Leaf number	Leaf area (cm^2)	Pseudostem length (cm)	Root number	Root length (cm)
0	15.68 ± 2.31 ^{bc}	4.33 ± 0.52 ^{ab}	21.79 ± 7.71	11.36 ± 1.88 ^{ab}	6.56 ± 2.07	4.87 ± 2.06 ^{ab}
0.1	16.94 ± 2.84 ^a	4.61 ± 0.50 ^a	18.70 ± 3.71	10.95 ± 1.84 ^{ab}	6.89 ± 1.87	5.56 ± 2.23 ^{ab}
0.5	14.49 ± 2.74 ^c	4.22 ± 0.67 ^{ab}	21.53 ± 6.16	9.39 ± 1.87 ^b	5.67 ± 1.61	5.22 ± 1.57 ^{ab}
1.0	15.57 ± 2.58 ^{bc}	3.50 ± 0.73 ^b	19.81 ± 5.22	9.58 ± 1.84 ^b	5.94 ± 1.39	3.90 ± 0.79 ^b
2.0	15.33 ± 2.39 ^{bc}	4.39 ± 0.78 ^{ab}	25.54 ± 5.97	10.52 ± 1.88 ^{ab}	7.17 ± 1.34	6.12 ± 1.88 ^a
3.0	16.33 ± 2.67 ^{ab}	4.22 ± 0.83 ^{ab}	21.55 ± 6.45	10.25 ± 2.01 ^{ab}	6.61 ± 1.58	4.72 ± 1.39 ^{ab}
4.0	—	—	—	—	—	—
F-test	350.717	26.005	7.537	164.862	13.889	11.854
CV (%)	42.13	45.35	49.35	42.74	46.18	49.92

Note: Values represent mean $\pm$ standard deviation (SD). Means followed by different lowercase letters within the same column are significantly different based on Duncan's Multiple Range Test (DMRT) at $p < 0.05$. A dash (—) indicates that no data were recorded due to complete mortality at the highest NaN_3

concentration.

3.3 Interpretation of NaN_3 -induced physiological responses

The physiological responses of *M. ornata* plantlets observed in this study, including changes in survival rate, growth traits, and photosynthetic pigments, reflect concentration-dependent effects of NaN_3 under *in vitro* conditions. However, the present study did not directly investigate the underlying biochemical or molecular mechanisms responsible for these responses. Therefore, any discussion of potential cellular or signaling pathways should be interpreted as contextual background derived from previous studies rather than as direct evidence from the current experiment.

The observed dose-dependent inhibitory and stimulatory trends are consistent with patterns commonly reported in chemical mutagenesis studies, where low concentrations may induce mild stress responses and higher

concentrations lead to cytotoxic effects. In the absence of direct mechanistic measurements, the current findings are best interpreted at the phenotypic and physiological levels, providing a descriptive foundation for future studies that integrate molecular, biochemical, or genetic analyses to elucidate the mechanisms underlying NaN_3 -induced responses in *Musa* species.

3.4 Changes in photosynthetic pigments

The contents of chlorophyll a, chlorophyll b, and total chlorophyll in *M. ornata* plantlets showed numerical variation across NaN_3 concentrations; however, some significant differences were observed differences were detected among treatments based on the mean-letter grouping (Table 4). Although the lowest mean values were observed at 0.5 mM NaN_3 , these differences could not be confirmed statistically. Similarly, carotenoid content remained statistically unchanged across all treatments.

Table 4. Effects of sodium azide (NaN_3) on photosynthetic pigment contents of *Musa ornata* plantlets.

NaN_3 (mM)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
0	0.257 ± 0.038^a	0.418 ± 0.167	0.675 ± 0.205	0.247 ± 0.063
0.1	0.227 ± 0.011^{ab}	0.410 ± 0.014	0.637 ± 0.025	0.226 ± 0.009
0.5	0.179 ± 0.024^b	0.318 ± 0.036	0.496 ± 0.059	0.177 ± 0.023
1.0	0.221 ± 0.040^{ab}	0.400 ± 0.063	0.621 ± 0.103	0.223 ± 0.038
2.0	0.239 ± 0.032^{ab}	0.401 ± 0.021	0.641 ± 0.046	0.230 ± 0.017
3.0	0.213 ± 0.065^{ab}	0.390 ± 0.119	0.603 ± 0.184	0.204 ± 0.072
4.0	–	–	–	–
F-test	17.909	9.989	12.875	13.326
CV (%)	46.27	47.64	46.79	46.98

Note: Data are expressed as mean $\pm$ standard deviation (SD). Different lowercase letters within the same column denote significant differences among treatments according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$. Pigment contents were determined spectrophotometrically after 16 weeks of culture.

Therefore, the observed variations in pigment contents should be interpreted as descriptive trends rather than definitive treatment effects. No SPAD measurements were included in the present study, and pigment-related responses were evaluated solely based on spectrophotometric quantification. Consequently, evidence regarding pigment efficiency or functional alterations of the photosynthetic apparatus remains inconclusive.

Although a numerical reduction in chlorophyll a was observed at certain NaN_3 concentrations, this pattern was not statistically significant and cannot be interpreted as reflecting changes in chlorophyll biosynthesis, magnesium partitioning, or photosynthetic efficiency. The unchanged levels of chlorophyll b and carotenoids further indicate that pigment composition remained largely stable within the tested concentration range. Overall, the present results provide descriptive information on pigment variation in response to NaN_3 exposure, and further studies integrating SPAD measurements and physiological or biochemical assessments would be

required to clarify functional implications.

Collectively, the results indicate that NaN_3 exposure was associated with numerical variations in pigment contents of *M. ornata* plantlets across treatments. However, the present study did not directly assess oxidative stress markers, reactive oxygen species production, or antioxidant responses. Therefore, the effects of NaN_3 on pigment dynamics are interpreted at a descriptive level, based solely on observed changes in chlorophyll and carotenoid contents. Any potential involvement of oxidative or photoprotective mechanisms cannot be confirmed within the scope of the current experimental design and should be addressed in future studies incorporating biochemical and molecular analyses.

3.5 Leaf color and visual parameters

Leaf color parameters of *M. ornata* plantlets, expressed in the CIELAB color space (L^* , a^* , and b^*), were quantified using a Linshang LS173 Colorimeter as described in Section 2.4. No statistically significant differences were detected among NaN_3 treatments for any of the

measured color parameters ($p > 0.05$; Table 5). Although numerical trends toward higher lightness (L^*) and lower a^* values were observed at 2.0 mM NaN_3 , these variations were not statistically significant and should therefore be interpreted as descriptive observations rather than definitive treatment effects.

3.6 Implications for ornamental banana improvement

The results of this study provide practical implications for the application of sodium azide (NaN_3) in ornamental banana improvement, particularly at the early screening stage under *in vitro* conditions. The concentration-dependent survival responses and the estimated LD_{30} (1.13 mM) and LD_{50} (2.08 mM) values establish quantitative reference points for selecting NaN_3 dosages that balance mutagenic effectiveness with plantlet viability.

Table 5. Leaf CIELAB color parameters (L^* , a^* , and b^*) of *Musa ornata* plantlets under different sodium azide (NaN_3) concentrations.

NaN_3 (mM)	L^*	a^*	b^*
0 (control)	42.24 ± 3.14	-9.87 ± 1.05	-9.36 ± 0.89
0.1	41.94 ± 3.09	-11.12 ± 0.80	21.19 ± 4.48
0.5	42.27 ± 2.90	-10.11 ± 0.79	24.14 ± 2.68
1	43.06 ± 1.70	-10.77 ± 0.61	24.70 ± 5.36
2	50.87 ± 4.36	-11.83 ± 0.23	24.25 ± 1.26
3	44.19 ± 0.88	-11.80 ± 0.36	30.90 ± 3.20
4	–	–	–
F-test (ANOVA)	39.83	41.85	10.04
CV (%)	43.78	43.71	48.59

Note: Values are presented as mean $\pm$ standard deviation (SD). Leaf color parameters were expressed in the CIELAB color space, where L^* indicates lightness, a^* represents the green (–) to red (+) axis, and b^* represents the blue (–) to yellow (+) axis. Analysis of variance (ANOVA) indicated no statistically significant differences among NaN_3 treatments ($p > 0.05$); therefore, no mean-letter grouping was assigned. F-test and coefficient of variation (CV) values are provided to indicate statistical variation and data dispersion.

Although several morphological and physiological traits exhibited numerical variation across NaN_3 concentrations, only traits showing statistically supported responses should be considered for practical application. In this context, survival rate and fresh weight responses provide the most reliable indicators for defining appropriate mutagen treatment ranges, whereas variations in root traits, pigment contents, and leaf color parameters should be regarded as

preliminary observations requiring further validation.

Therefore, the primary implication of this study lies in providing baseline dosage guidelines and a phenotypic screening framework for future mutation breeding programs in *M. ornata*. Subsequent studies incorporating long-term growth assessment, heritability analysis, and molecular characterization will be essential to confirm whether NaN_3 -induced variations observed at the

in vitro stage can be translated into stable and commercially valuable ornamental traits.

4. Conclusions

This study provides a systematic assessment of the morphological and physiological responses of *M. ornata* plantlets to sodium azide (NaN_3) mutagenesis under *in vitro* conditions. Survival rate declined in a clear concentration-dependent manner, allowing the estimation of LD_{30} (1.13 mM) and LD_{50} (2.08 mM), which serve as practical reference doses for balancing mutagen exposure and plantlet viability. Among the evaluated traits, fresh weight showed a statistically significant increase at low NaN_3 concentration (0.1 mM), whereas leaf number, pseudostem length, root traits, photosynthetic pigments, and leaf color parameters exhibited only numerical variation without consistent statistical support. These results indicate that most observed responses should be interpreted descriptively rather than as definitive treatment effects.

Overall, the primary contribution of this study lies in establishing species-specific lethal dose thresholds and providing baseline phenotypic screening information for NaN_3 application in *M. ornata*. The findings support the use of NaN_3 as a preliminary mutagenic tool for generating phenotypic variability at the *in vitro* stage, while emphasizing that further studies involving long-term growth evaluation, heritability assessment, and molecular validation are required to determine the stability and breeding value of the induced variations.

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