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SALT-TOLERANT RICE CULTIVARS' SENSITIVITY TO SODIUM AZIDE REVEALED BY LD$_{50}$ ANALYSIS

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SUMMARY

Chemical mutagenesis is an important approach in rice (*Oryza sativa* L.) improvement, as it can develop novel genetic variation for breeding programs. Sodium azide (SA) is one of the most effective chemical mutagens used in cereals; however, information on the sensitivity of Indonesian salt-tolerant rice cultivars to SA remains limited. This study aimed to determine the LD$_{50}$ values of SA in two salt-tolerant cultivars (Biosalin-1-Agritan and Biosalin-2-Agritan) and one high-yielding cultivar (Inpari 32 HDB). Three rice cultivars sustained exposure to varying SA doses, examining their germination traits, seedling vigor, and their survival responses through median lethal doses (LD$_{50}$) values. The results revealed distinct genotype-dependent responses. The rice cultivars Biosalin-1-Agritan and Biosalin-2-Agritan emerged with LD$_{50}$ values of 8.30 and 9.20 mM, respectively, indicating moderate sensitivity to SA. In contrast, cultivar Inpari 32 HDB demonstrated the highest tolerance with LD$_{50}$ (17.00 mM) with better performance in germination, synchrony, and growth across the SA doses. The root and shoot growth showed inhibition in a dose-dependent manner in all rice genotypes; however, the extent of inhibition was more severe in the Biosalin cultivars, particularly with doses of ≥6 mM. The rice genotypes screening provides a considerable baseline before chemical mutagenesis through SA doses in rice breeding programs.

Keywords: Rice (*O. sativa* L.), mutagenesis, sodium azide, screening, sensitivity, seed viability, seed vigor

Key findings: This study identified genotype-specific responses to sodium azide in rice (*O. sativa* L.), revealing salt-tolerant cultivars were more sensitive to chemical mutagenesis than a popular high-yielding cultivar. The LD$_{50}$ values provide critical baseline data for optimizing mutagenesis protocols in rice breeding programs.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the most essential staple food for more than half of the world's population, particularly in Asia (Fukagawa and Ziska, 2019), including Indonesia (Sutardi *et al.*, 2023). However, rice cultivation faces considerable threats from soil salinity, especially in coastal and arid regions (Sackey *et al.*, 2025). In saline-prone regions, the accumulation of soluble ions (Na^+, Ca^+, K^+, and Mg^{2+}) in the root zone inhibits plant growth and development through ionic imbalance, osmotic stress, oxidative stress, and, eventually, reduced nutrient uptake (Atta *et al.*, 2023). In addressing these challenges, the development of salt-tolerant rice cultivars has become a key objective of modern rice breeding (Herwibawa *et al.*, 2024; Waheed *et al.*, 2024).

In managing salinity constraints, the National Research and Innovation Agency (BRIN), Indonesia, developed two salt-tolerant rice cultivars, Biosalin-1-Agritan and Biosalin-2-Agritan. These cultivars' official release was under the Decree of the Minister of Agriculture of the Republic of Indonesia No. 894/HK.540/C/06/2020 and No. 895/HK.540/C/06/2020, respectively. These rice cultivars succeeded in their development through mutation breeding using gamma irradiation of embryogenic callus cultures, deriving Biosalin-1-Agritan from Ciherang (22.468 Gy) and Biosalin-2-Agritan from Inpari 13 (23.124 Gy). Cultivar Inpari 32 HDB is also the most widely cultivated high-yielding rice cultivar in Indonesia. These improved rice cultivars demonstrated enhanced seedling-stage tolerance to salinity and are currently being promoted for cultivation in the marginal lands.

In our previous study, the salt tolerance assessment in rice cultivars Biosalin-1-Agritan and Biosalin-2-Agritan under varying NaCl doses (0–50 ppt) revealed both cultivars maintained growth up to 10 ppt; however, they experienced significant reduction in growth and development with higher salinity (Herwibawa *et al.*, 2025). These findings disclosed the scope for further improvement through advanced breeding methods to enhance the rice genotypes resilience under extreme salinity stress conditions.

Mutation breeding remains one of the most effective tools for generating considerable variations in plant populations (De-Oliveira and Vanavichit, 2023). Among these mutagenic agents, sodium azide (SA) has become one of the most efficient chemical mutagens for cereals because it induces point mutations, mainly C → T changes (Liu *et al.*, 2025), at a high frequency with relatively low chromosomal aberrations (Punia *et al.*, 2022). SA has been applicable in various crops, with reported LD_{50} values varying considerably across species, e.g., 3.23–3.69 mM in garlic (Mahajan *et al.*, 2015), 1.60–2.69 mM in pigeon pea (Chaudhary *et al.*, 2021), 4 mM in wheat (Türkoğlu *et al.*, 2022), 8 mM in fenugreek (Khan *et al.*, 2024), 23.3 mM in safflower (Mirza *et al.*, 2024), and 0.047 mM in mungbean (Ali *et al.*, 2024). The aromatic upland rice, cv. Inpago Unsoed 1 showed the LD_{50} of 8.84 mM (Herwibawa and Kusmiyati, 2017), highlighting the genotype-specific nature of LD_{50} values. However, information remains limited for Indonesian rice cultivars, particularly those bred for salt tolerance, such as Biosalin-1-Agritan and Biosalin-2-Agritan (Herwibawa *et al.*, 2025). Determining the appropriate mutagen dose is crucial in chemical mutagenesis because excessive doses can be lethal (Ke *et al.*, 2019). The LD_{50}, defined as the dose causing a 50% reduction in seed germination (Nilahayati *et al.*, 2024), represents a critical threshold in mutation breeding, balancing mutagenic efficiency with plant survival.

Although the development of salt-tolerant rice cultivars was to cope with saline soils, it is still unknown whether their tolerance to salinity has an association with tolerance or sensitivity to chemical mutagens. This gap needs addressing to provide a better comprehension of genotype-specific mutagenic responses. The presented study aimed to determine the LD_{50} values of sodium azide for three improved rice cultivars, Biosalin-1-Agritan, Biosalin-2-Agritan, and Inpari 32 HDB. Cultivar Inpari 32 HDB is a classified irrigated lowland rice cultivar that has been widely adopted by the farming community due to its agronomic stability and disease resistance. Specifically, the objective of this study was to compare the genotypic sensitivity of salt-tolerant and high-yielding rice cultivars to sodium azide and to provide baseline information for optimizing future mutagenesis programs. Establishing accurate LD_{50} thresholds seeks to guide future mutagenesis programs, especially for improving salt stress tolerance in elite rice cultivars suited to saline-prone areas.

MATERIALS AND METHODS

This study used three rice (*O. sativa* L.) cultivars, Biosalin-1-Agritan, Biosalin-2-Agritan, and Inpari 32 HDB. For each rice cultivar, the experiment established 11 treatments. One treatment served as the untreated control (0 mM sodium azide-SA), while the remaining 10 treatments bore exposure to SA doses of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mM. Germinating rice seeds proceeded on moist tissue paper. Since the dose range did not yield the LD_{50} value for Inpari 32 HDB, the study used an extended series of SA doses. For the 0–30 mM treatments, rice seed germination continued on moist rice straw paper. For this extended test, germination of seeds on moist rice straw paper occurred instead of tissue paper, as straw paper provides better aeration during prolonged germination assays and ensures more accurate LD_{50} estimation. Each treatment comprised 100 seeds, with three replicates. Seeds soaked in 0.1 M KH_2PO_4 buffer and pH 3.5 contained the respective SA doses for six hours at approximately 25 °C (Herwibawa and Kusmiyati, 2017). After treatment, the seeds received thorough rinsing under running water for 1.5 h to remove residual sodium azide (Shin and Jeung, 2011). Subsequently, the rice seed achieved germination under laboratory conditions, with daily watering. The experiment had a completely randomized design (CRD) arrangement.

Rice seeds become germinated when the radicle protrusion reaches at least 2 mm (Prasad *et al.*, 2023). Germination percentage (GP) recording was on the fifth and 14th days after sowing. The GP calculation used the formula GP (%) = (NT/N) × 100, where NT was the number of germinated seeds and N is the total number of seeds (Marcu *et al.*, 2013). Mean germination time (MGT) computation was MGT = Σfx/Σf, where f was the number of seeds that germinated on day x (Orchard, 1977). Recording the first day of germination (FDG) and last day of germination (LDG) also took place (Al-Mudaris, 1998). The determination of the coefficient of velocity of germination (CVG) comprised the CVG = ([N1/T1 + N2/T2 +…+ Nx/Tx]/ [N1 + N2 + … + Nx]) × 100, where N was the number of seeds germinated each day, and T was the corresponding day of germination (Jones and Sanders, 1987). Germination rate index (GRI) calculation was the GRI = $G_1/1 + G_2/2 + \ldots + G_x/x$, with G_1, G_2,…, G_x being germination percentages multiplied by 100 on each respective day (Esechie, 1994). Calculating germination index (GI) was the GI = $(10 \times n_1) + (9 \times n_2) + \ldots + (1 \times n_{10})$, where n_1 to n_{10} represent the number of seeds germinated each day (Arnold *et al.*, 1991). Morphological parameters' measurement included seedling height (SH) and root length (RL), with seedling height measured from the base of the stem to the tip of the longest leaf. Meanwhile, root length measurement began from the base of the stem to the tip of the longest root (Salsinha *et al.*, 2023).

Using rice seeds' germination data helped estimate the LD_{50}, which represents the dose of sodium azide that reduced the germination by 50% in the seed population. The LD_{50} value, as determined, used the drc package in R, which applies nonlinear regression models for dose-response analysis. A three-parameter log-logistic model (LL.3) fitted to the data accurately described the sigmoidal response curve. Then, calculating the effective dose (ED_{50}) utilized the ED function, with the delta method used for interval estimation. Data processing and visualization continued in R version 4.5.0 for Windows (https://cran.r-project.org/bin/windows/base/) via RStudio version 2025.05.0+496 (https://posit.co/download/rstudio-desktop/).

RESULTS

Survival response and LD_{50} estimation

In determining the phytotoxic effects of sodium azide (SA) on rice, three Indonesian cultivars with different stress backgrounds, Biosalin-1-Agritan and Biosalin-2-Agritan (salt-tolerant cultivars) and Inpari 32 HDB (a widely cultivated conventional cultivar), sustained exposure to enhanced SA doses, comparing the SA treatments for various physiological parameters. Dose-response assays revealed contrasting levels of SA sensitivity among the rice genotypes. Survival rates decreased sigmoidally with increasing SA doses (Figure 1). The LD_{50} values differed markedly among cultivars, allowing classification of their sensitivity to SA. Biosalin-1-Agritan and Biosalin-2-Agritan showed LD_{50} values of 8.30 and 9.20 mM, respectively. These values fall within the moderate sensitivity range (8–10 mM) and are comparable to the LD_{50} of 8.84 mM previously reported for the aromatic upland cultivar Inpago Unsoed 1 (Herwibawa and Kusmiyati, 2017). By contrast, Inpari 32 HDB exhibited a much higher LD_{50} of 17.00 mM, placing it in the tolerant category (≥15 mM). For reference, the classification of cultivars with LD_{50} values is as follows: below 5 mM are generally highly sensitive, those within 5–7 mM are sensitive, 8–10 mM are moderately sensitive, 11–14 mM are moderately tolerant, and ≥15 mM are tolerant.

Seedling morphology under SA stress

Visual inspection of the rice seedling morphology corroborated the survival trends. Seedlings grown under SA doses ranging from 0 to 10 mM displayed progressive growth inhibition for shoot and root systems (Figure 2A). Biosalin rice cultivars exhibited severe developmental arrest at ≥6 mM, with shorter shoots and undeveloped roots. Cultivar Inpari-32 HDB maintained relatively intact morphology at equivalent doses. Further exposure of the rice cultivar Inpari 32 HDB to higher SA doses (12–30 mM) revealed a gradual decline in growth and development and, eventually, in seedling size, culminating in almost complete growth suppression at 27–30 mM (Figure 2B).

Sodium azide effect on germination and timing

Sodium azide significantly affected and altered the rice seed germination dynamics in a dose-dependent manner. Germination percentage (GP) at the fifth day declined sharply as SA levels increased, particularly in the cultivar Biosalin-1-Agritan, and showed nearly complete suppression of early germination beyond 3 mM (Figure 3A). With the final count (day 14), the GP also decreased in all the rice cultivars, although cultivar Inpari 32 HDB retained relatively higher values across the entire SA levels tested (Figure 3B). Delay in rice seeds' germination manifested both on the first day of germination (FDG) and the last day of germination (LDG), which also increased with SA doses across all the genotypes (Figure 3C, 3D). The germination delay was most notable in rice cultivar Biosalin-1-Agritan, indicating reduced synchrony and vigor at the early seedling establishment under SA stress conditions.

Germination vigor indices

The germination vigor indices progressively declined with increasing SA doses. Mean germination time (MGT) increased in all the cultivars, with the cultivar Biosalin-1-Agritan consistently recorded with the slowest germination rate at most doses (Figure 4A). Conversely, the coefficient of velocity of germination (CVG) showed a steep decline even at low SA doses, plateauing beyond 6 mM in all the rice genotypes (Figure 4B). Both germination index (GI) and germination rate index (GRI) decreased in a dose-dependent manner, and the cultivar Inpari 32 HDB considerably maintained the higher values than both Biosalin cultivars throughout the SA gradient (Figure 4C, 4D), suggesting greater resilience in maintaining germination performance within stress conditions.

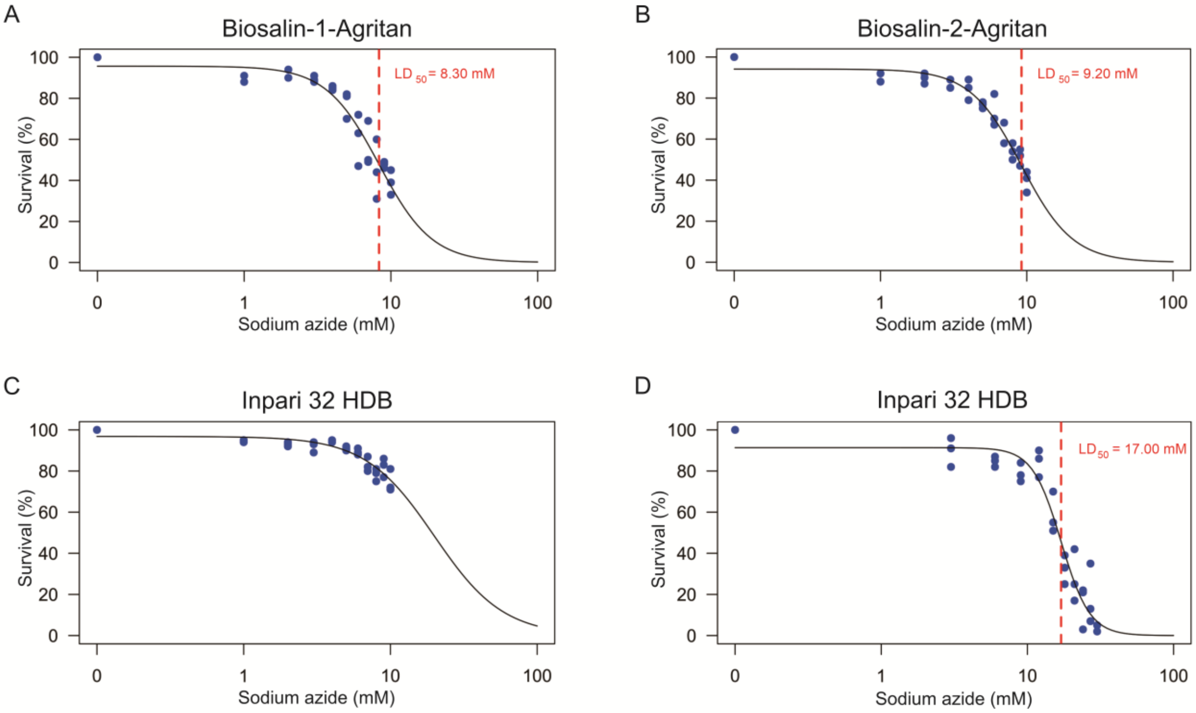

Figure 1. Dose-response curves of seedling survival in three Indonesian rice cultivars exposed to sodium azide (SA). A) Biosalin-1-Agritan, a salt-tolerant cultivar, exhibited an LD_{50} of 8.30 mM; B) Biosalin-2-Agritan, also salt-tolerant, showed an LD_{50} of 9.20 mM; C) Inpari 32 HDB, a conventional cultivar, did not reach LD_{50} within the initial dose range (0–10 mM); therefore, the LD_{50} line is not visible in this panel; and D) Inpari 32 HDB showed an LD_{50} of 17.00 mM. Each curve represents a nonlinear regression fit describing the sigmoidal dose-response relationship.

Shoot and root development

In rice genotypes, various SA treatments compromised the shoot and root development. Shoot height (SH) decreased with gradually increasing doses of SA, and the significant reduction appeared at ≥4 mM across all rice cultivars (Figure 5A). The cultivar Inpari 32 HDB displayed the highest shoot with the low to moderate SA doses, although the said advantage diminished with exposure to higher SA doses. Root length (RL) exhibited higher variability, especially with the doses between 0 and 5 mM, but sharply declined at ≥6 mM across all rice cultivars (Figure 5B). The rapid root inhibition highlighted the highest sensitivity of root tissues to SA toxicity. The results further indicated sodium azide induces dose-dependent reduction in seed viability, germination efficiency, and early seedling development across all rice cultivars. The entire rice cultivars experienced stress-related impairment, and the conventional cultivar Inpari 32 HDB demonstrated comparatively higher tolerance for survival, germination, and seedling growth than salt-tolerant Biosalin cultivars.

DISCUSSION

The presented results provided new insights through the varied responses of Indonesian rice cultivars to sodium azide (SA) exposure, revealing genotype-dependent variation in terms of phytotoxicity tolerance. Ikhajiagbe and Omoregie’s (2020) findings demonstrated that SA treatment can improve growth and yield-related traits under salinity stress conditions, with its broader application constrained by genotype-specific phytotoxic effects. In the latest study, dose-response analysis revealed a sigmoidal decline in survival with increasing SA doses across all cultivars, underscoring the importance of determining genotype-specific LD_{50} thresholds to balance the mutagenic efficiency. The LD_{50} values observed for the Biosalin rice cultivars enunciated moderate sensitivity, aligning with previous findings on the mutagenesis in rice for drought tolerance (Herwibawa and Kusmiyati, 2017). In contrast, cultivar Inpari 32 HDB

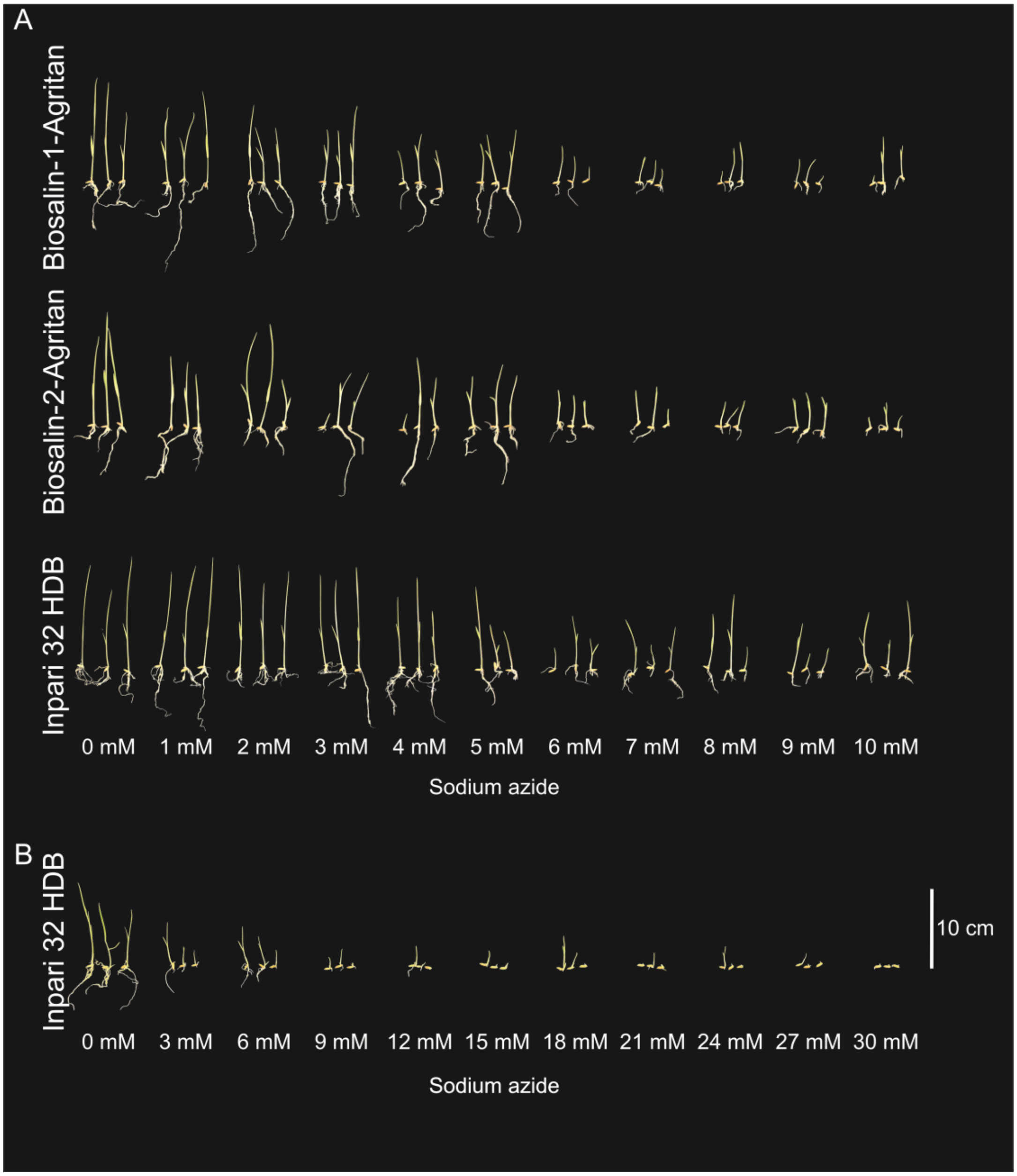

Figure 2. Visual representation of rice seedling growth under different doses of sodium azide (SA). A) Seedling morphology of Biosalin-1-Agritan, Biosalin-2-Agritan, and Inpari 32 HDB germinated on moist tissue paper under SA doses ranging from 0 to 10 mM, and B) Morphological response of Inpari 32 HDB seedlings germinated on moist rice straw paper under an extended SA dose range from 0 to 30 mM (in 3 mM increments). Scale bar = 10 cm.

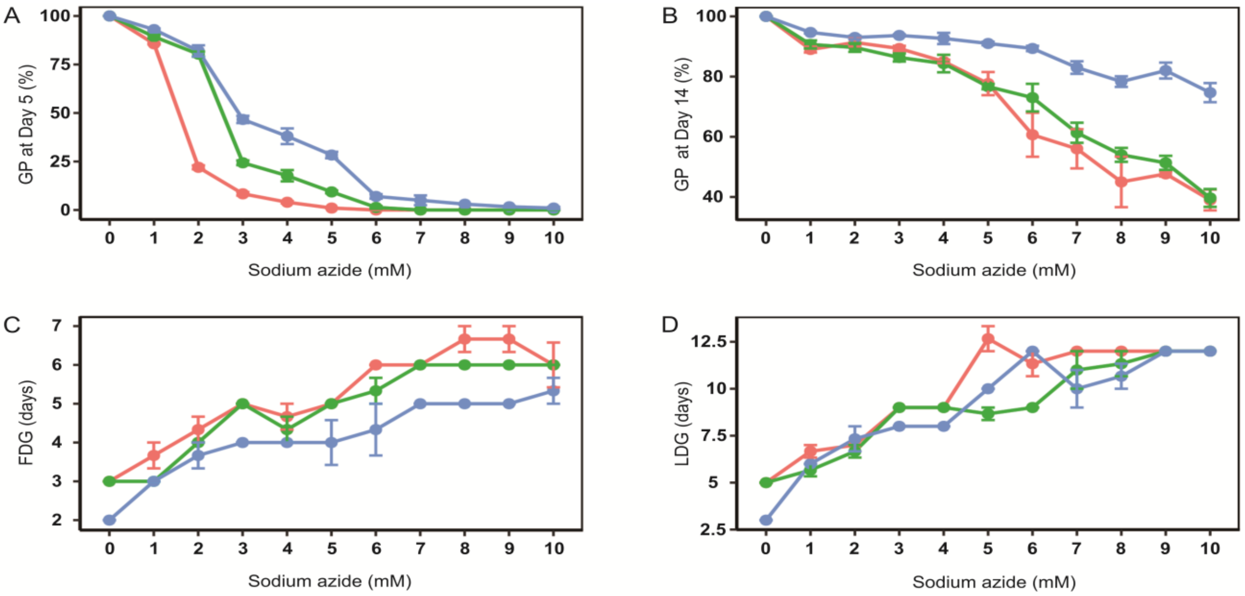

Figure 3. Line plots showing the effects of different sodium azide doses (0–10 mM) on seed germination parameters of three rice cultivars: Biosalin-1-Agritan (red), Biosalin-2-Agritan (green), and Inpari 32 HDB (blue). A) Germination percentage on day 5 (GP, %, first count); B) Germination percentage on day 14 (GP, %, final count); C) First day of germination (FDG, days); and D) Last day of germination (LDG, days). Data are presented as means ± standard error.

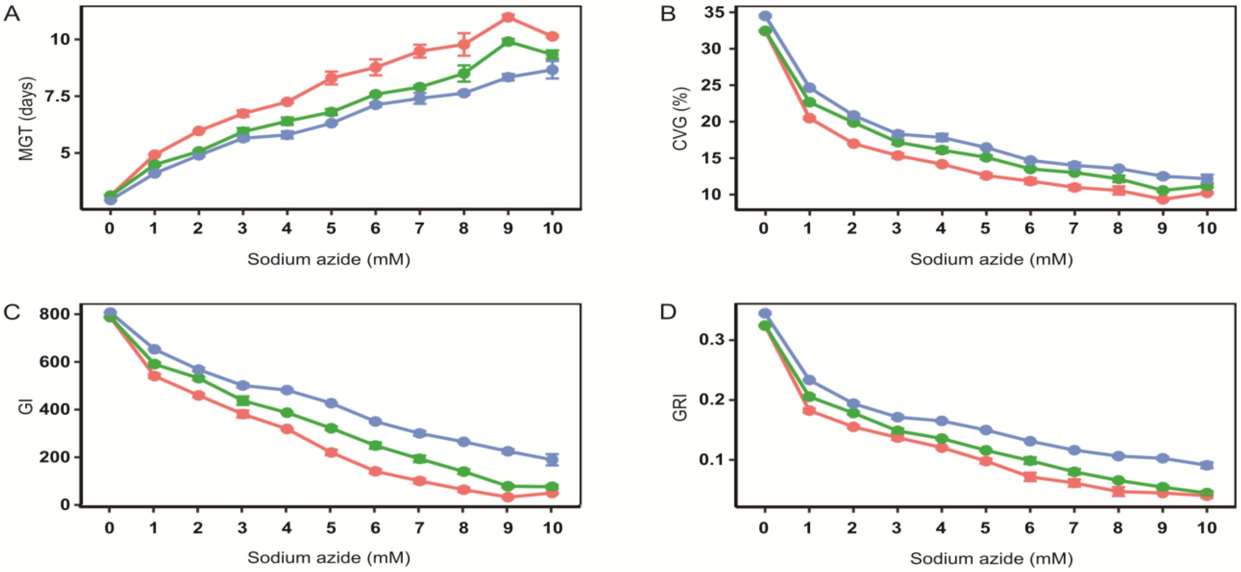

Figure 4. Line plots showing the effects of different sodium azide doses (0–10 mM) on seed germination vigor indices of three rice cultivars: Biosalin-1-Agritan (red), Biosalin-2-Agritan (green), and Inpari 32 HDB (blue). A) Mean germination time (MGT, days); B) Coefficient of velocity of germination (CVG, %); C) Germination index (GI); and D) Germination rate index (GRI). Data are presented as means ± standard error.

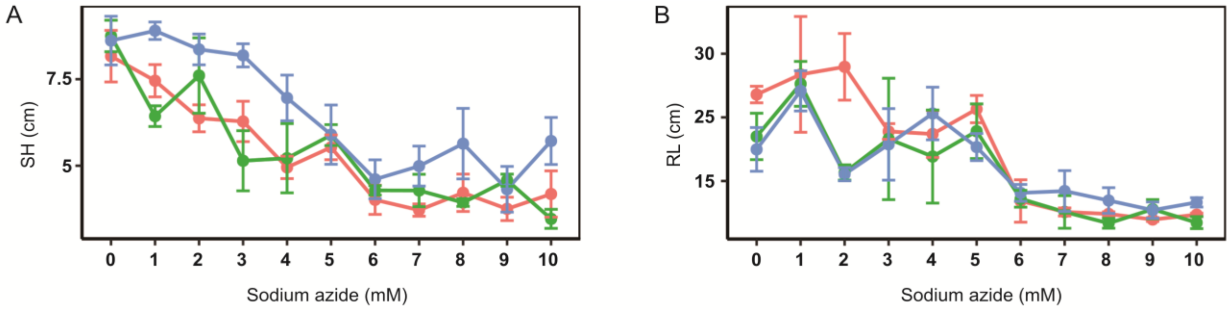

Figure 5. Line plots showing the effects of different sodium azide doses (0–10 mM) on seedling growth parameters of three rice cultivars: Biosalin-1-Agritan (red), Biosalin-2-Agritan (green), and Inpari 32 HDB (blue). A) Shoot height (SH, cm) and B) Root length (RL, cm). Data are presented as means ± standard error.

exhibited a notably higher LD_{50}, suggesting a greater capacity to mitigate the toxic effects of SA. However, since this study did not investigate DNA repair pathways or antioxidative enzyme activities, no conclusions can be drawn about these mechanisms. The observed differences show more conservative interpretations of genotype-specific physiological responses (Gómez *et al*., 2019; Al-Aboudi *et al.*, 2023).

Morphological assessment received corroboration from these survival patterns, as the shoot and root development reached considerable inhibition in the Biosalin rice cultivars compared with the cultivar Inpari 32 HDB. The inhibition of cytochrome oxidase and disruption of mitochondrial membrane potential by SA resulted in impaired ATP biosynthesis, which, in turn, slowed down the cell division and elongation and, eventually, led to reduced shoot and root growth and development (Srivastava *et al.*, 2011). Reports of similar findings have also come out for fenugreek (Siddiqui *et al.*, 2007), watermelon and moringa (AbdulRahaman *et al.*, 2018), onions (Adeoti *et al.*, 2021), sesame (Weldemichael *et al.*, 2021), and Faber's cymbidium (Wu *et al.*, 2024). The abrupt decline in root length at doses ≥6 mM across all rice cultivars further highlighted the sensitivity of root tissues, and the same may be due to the high permeability and rapid uptake of SA by roots. Study results are consistent with Calvo-Polanco *et al.* (2021), whose findings also showed root water transport in *Arabidopsis* was highly dependent on functional barriers and aquaporins, which were disrupted by SA. These results supported the hypothesis that mutagen-induced root inhibition was a reliable indicator of systemic phytotoxicity.

Biosalin rice cultivars' recording gave delayed and desynchronized germination patterns, further reflecting their susceptibility to SA stress conditions. Lower germination percentage at the first (day 5) and final (day 14) counts, combined with increased MGT and LDG, suggested compromised seed metabolic activity and embryo viability. However, such types of delays indicated a likely association with SA-induced disruptions in cellular metabolism, particularly the inhibition of cytochrome c oxidase and ATP synthesis, which impaired the energy-dependent processes essential for timely and synchronized germination (Sayed *et al.*, 2025). The cultivar Inpari 32 HDB, with superior performance for germination parameters, including CVG, GI, and GRI, underscores its robust seed vigor and potential suitability as a model for mutagenesis screening.

Interestingly, despite being selected for salt tolerance, rice cultivars Biosalin-1-Agritan and Biosalin-2-Agritan showed greater susceptibility to SA than the conventional cultivar Inpari 32 HDB. This suggests a potential trade-off between adaptations to salinity stress and tolerance to chemical mutagens. As mechanisms, such as ion exclusion and osmotic adjustment, confer advantages under saline conditions, they do not necessarily protect against mutagens like SA, which primarily impair DNA integrity and mitochondrial energy metabolism. Similar genotype-dependent trade-offs have been evident in other crops (Dwivedi *et al.*, 2021), supporting the need to adjust mutagen doses for stress-tolerant genotypes. The mutagenic effects typically follow the specific dose-response relationship that can be linear or non-linear, with defined threshold or non-threshold effects. Although non-genotoxic agents seemed generally to have threshold doses, which indicates that some genotoxic carcinogens may also result in threshold responses (Georgieva and Vassileva, 2023), it suggests the complexity of predicting mutagenic outcomes across the genotypes.

The current findings highlighted the importance of genotype screening before a large-scale mutation breeding program. Although the cultivar Inpari 32 HDB appears more resilient, the safety margins for SA application remain narrow, and doses above 10 mM may cause irreversible damage even in rice-tolerant cultivars. However, some limitations exist in extrapolating these findings. The mechanisms underlying the differential responses received no analysis in the presented study. Future research should integrate molecular approaches, such as transcriptomic and metabolomic profiling, to elucidate the genetic and biochemical bases of SA detoxification and tolerance. This could lead to the development of rice genotypes tailored for high-efficiency mutagenesis and improved resilience to genotoxic stress conditions.

CONCLUSIONS

This study demonstrated that sodium azide induces genotype-specific phytotoxic effects in rice, and the salt-tolerant rice cultivars Biosalin-1-Agritan and Biosalin-2-Agritan showed moderate sensitivity, while the cultivar Inpari 32 HDB exhibited greater tolerance. The LD_{50} values provided critical thresholds for optimizing SA dosage in mutation breeding. Despite genotypes' tolerance to salinity, Biosalin cultivars emerged more vulnerable to SA-induced reduction in germination and seedling vigor. These results suggest that tolerance to salinity stress does not necessarily confer tolerance to chemical mutagens, indicating a potential trade-off between stress adaptation and mutagen sensitivity. The results underscored the importance of genotypes' screening before mutagen application and supported the use of conventional cultivar Inpari 32 HDB as a potential model for high-efficiency mutagenesis. Accurate determination of LD_{50} thresholds is therefore essential when planning chemical mutagenesis, particularly for stress-tolerant genotypes. Future work should integrate molecular approaches, such as transcriptomics and metabolomics, to better elucidate the mechanisms of SA detoxification and mutagen tolerance.

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REFERENCES

AbdulRahaman AA, Afolabi AA, Zhigila DA, Oladele FA, Al Sahli AA (2018). Morpho-anatomical effects of sodium azide and nitrous acid on *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Cucurbitaceae) and *Moringa oleifera* Lam. (Moringaceae). *Hoehnea* 45(2): 225–237. http://dx.doi.org/10.1590/2236-8906-26/2017.

Adeoti OM, Sodiq ZO, Komolafe KA (2021). Effects of chemical mutagen (sodium azide) on onion grown in organic and inorganic fertilized soil. *Res. Ecol.* 3(2): 5–9. https://doi.org/10.30564/re.v3i2.2908.

Al-Aboudi AAAA, Hamdallah MS, Musa AKJ (2023). Effect of sodium azide on some physiological traits of genotypes of rice (*Oryza sativa* L.) under different salinity levels. *Euphrates J. Agric. Sci*. 15(1): 136–152.

Ali SMDB, Konjengbam NS, Ahmad F, Sanasam S, Kumawat R (2024). Ascertaining lethal dose 50 (LD$_{50}$) and simultaneous effect of ethyl methane sulphonate (EMS) and sodium azide (SA) on seedling characters in mungbean genotypes 'Pusa 1031' and 'Pusa 1431'. *Legume Res.* 47: 1929–1935. https://doi.org/10.18805/LR-5255.

Al-Mudaris MA (1998). Notes on various parameters recording the speed of seed germination. *J. Agric. Trop. Subtrop.* 99: 147–154.

Arnold RLB, Fenner M, Edwards PJ (1991). Changes in germinability, ABA content, and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench induced by water stress during grain filling. *New Phytol.* 118: 339–347. https://doi.org/10.1111/j.1469-8137.1991.tb00986.x.

Atta K, Mondal S, Gorai S, Singh AP, Kumari A, Ghosh T, Roy A, Hembram S, Gaikwad DJ, Mondal S, Bhattacharya S, Jha UC, Jespersen D (2023). Impacts of salinity stress on crop plants: Improving salt tolerance through genetic and molecular dissection. *Front. Plant Sci.* 14: 1241736. https://doi.org/10.3389/fpls.2023.1241736.

Calvo-Polanco M, Ribeyre Z, Dauzat M, Reyt G, Hidalgo-Shrestha C, Diehl P, Frenger M, Simonneau T, Muller B, Salt DE, Franke RB, Maurel C, Boursiac Y (2021). Physiological roles of Casparian strips and suberin in the transport of water and solutes. *New Phytol.* 232(6): 2295–2307. https://doi.org/10.1111/nph.17765.

Chaudhary L, Sharma R, Kumar M (2021). Estimation of LD50 and effect of sodium azide on germination and seedling parameters of different cultivars of *Cajanus cajan* (L.) Millspaugh. *Toxicol. Environ. Health Sci.* 13: 279–285. https://doi.org/10.1007/s13530-021-00105-6.

De-Oliveira AC, Vanavichit A (2023). Editorial: Generating useful genetic variation in crops by induced mutation, Volume III. *Front. Plant Sci.* 14: 1301977. https://doi.org/10.3389/fpls.2023.1301977.

Dwivedi SL, Reynolds MP, Ortiz R (2021). Mitigating tradeoffs in plant breeding. *iScience* 24(9): 102965. https://doi.org/10.1016/j.isci.2021.102965.

Esechie HA (1994). Interaction of salinity and temperature on the germination of sorghum. *J. Agron. Crop Sci.* 172(3): 194–199. https://doi.org/10.1111/j.1439-037X.1994.tb00166.x.

Fukagawa NK, Ziska LH (2019). Rice: Importance for global nutrition. *J. Nutr. Sci. Vitaminol.* 65: S2–S3. https://doi.org/10.3177/jnsv.65.S2.

Georgieva M, Vassileva V (2023). Stress management in plants: Examining provisional and unique dose-dependent responses. *Int. J. Mol. Sci.* 24(6): 5105. https://doi.org/10.3390/ijms24065105.

Gómez D, Hernández L, Martínez J, Escalante D, Zevallos BE, Yabor L, Trethowan R, Beemster GTS, Sershen, Lorenzo JC (2019). Sodium azide mutagenesis within temporary immersion bioreactors modifies sugarcane in vitro micropropagation rates and aldehyde, chlorophyll, carotenoid, and phenolic profiles. *Acta Physiol. Plant.* 41: 114. https://doi.org/10.1007/s11738-019-2911-0.

Herwibawa B, Kusmiyati F (2017). Mutagenic effects of sodium azide on the germination in rice (*Oryza sativa* L. cv Inpago Unsoed 1). *J. Agroteknol.* 7: 9–14. http://doi.org/10.24014/ja.v7i2.2759.

Herwibawa B, Kusmiyati F, Arafat S, Irawan AF, Anasrullah A (2025). Limited salt tolerance of Indonesian rice varieties Biosalin-1-Agritan and Biosalin-2-Agritan at early seedling stage under NaCl stress. *Trends Sci.* 22: 9305. https://doi.org/10.48048/tis.2025.9305.

Herwibawa B, Lekklar C, Chadchawan S, Buaboocha T (2024). Association of a specific *OsCULLIN3c* haplotype with salt stress responses in local Thai rice. *Int. J. Mol. Sci.* 25: 1040. https://doi.org/10.3390/ijms25021040.

Ikhajiagbe B, Omoregie UE (2020). Growth, yield, genetic parameters, and random amplified polymorphic DNA (RAPD) of five rice varieties treated with sodium azide and sown under different saline conditions. *Bull. Natl. Res. Cent.* 44: 89. https://doi.org/10.1186/s42269-020-00344-6.

Jones K, Sanders D (1987). The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. *J. Seed Technol.* 11: 97–102.

Ke C, Guan W, Bu S, Li X, Deng Y, Wei Z, Wu W, Zheng Y (2019). Determination of absorption dose in chemical mutagenesis in plants. *PLoS One* 14: e0210596. https://doi.org/10.1371/journal.pone.0210596.

Khan R, Punia SS, Dalip, Ram M, Gupta D, Bhatt B, Ahmad S, Dheer M, Rajput SS, Kumawat GL (2024). Determination of lethal dose (LD$_{50}$) and sensitivity of fenugreek (*Trigonella* *foenum-graecum*) to sodium azide for induction of mutation. *Indian J. Agric. Sci.* 94: 440–443. https://doi.org/10.56093/ijas.v94i4.134877.

Liu C, Frascarelli G, Stec AO, Heinen S, Lei L, Wyant SR, Legg E, Spiller M, Muehlbauer GJ, Smith KP, Fay JC, Morrell PL (2025). Sodium azide mutagenesis induces a unique pattern of mutations. *PLoS Genet.* 21: e1011634. https://doi.org/10.1371/journal.pgen.1011634.

Mahajan V, Devi S, Khar A, Lawande KE (2015). Studies on mutagenesis in garlic using chemical mutagens to determine lethal dose (LD_{50}) and create variability. *Indian J. Hortic.* 72: 289–292. https://doi.org/10.5958/0974-0112.2015.00055.9.

Marcu D, Damian G, Cosma C, Cristea V (2013). Gamma rays effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays* L.). *J. Biol. Phys.* 39: 625–634. https://doi.org/10.1007/s10867-013-9322-z.

Mirza S, Mahamune SE, Thorat SB (2024). The optimal LD50 gamma ray and sodium azide-induced mutagenesis in the PBNS-86 variety safflower. *J. Res. Appl. Sci. Biotechnol.* 3:116–119. https://doi.org/10.55544/jrasb.3.2.19.

Nilahayati N, Handayani RS, Nazimah N, Harahap MSA, Irawan G, Rosmaina R (2024). Determination of lethal dose 50 for induced mutagenesis in soybean [*Glycine max* (L.) Merril] cv. Gepak Kuning through ethyl methane sulfonate mutagen. *Agric. Sci. Dig.* 44: 892–897. https://doi.org/10.18805/ag.DF-544.

Orchard T (1977). Estimating the parameters of plant seedling emergence. *Seed Sci. Technol.* 5: 61–69.

Prasad MCT, Kodde J, Angenent GC, Hay FR, McNally KL, Groot SPC (2023). Identification of the rice *Rc* gene as a main regulator of seed survival under dry storage conditions. *Plant Cell Environ.* 46: 1962–1980. https://doi.org/10.1111/pce.14581.

Punia S, Singh M, Grover G (2022). Tilling: A reverse genetics technique. In: C. Tiwari (Ed.), Current Innovations in Genetics and Plant Breeding. Integrated Publications, Delhi, India. https://doi.org/10.22271/int.book.117.

Sackey OK, Feng N, Mohammed YZ, Dzou CF, Zheng D, Zhao L, Shen X (2025). A comprehensive review on rice responses and tolerance to salt stress. *Front. Plant Sci.* 16: 1561280. https://doi.org/10.3389/fpls.2025.1561280.

Salsinha YCF, Rini DS, Indradewa D, Rachmawati D, Alam T, Purwestri YA (2023). Exogenously applied *Casuarina equisetifolia* leaf extracts act as an osmoprotectant on proline accumulation under drought stress in local rice from Indonesia. *Front. Plant Sci.* 14: 1210241. https://doi.org/10.3389/fpls.2023.1210241.

Sayed AA, Elsayh E, Afifi H, Abdalgaleel MM, Abd Elaziz YSG, Barakat AM, Ahmed EA, El-Habashy S, Abdel Mageed YA, Omira GAM, El-Khayat LAS, El-Nemr MA, Salim NS, Arafa RN (2025). Effect of sodium azide mutagen on lavender plant salinity tolerance. *J. Harbin Eng. Univ.* 46(5): 122–151.

Shin YS, Jeung JU (2011). Genetic diversity estimation of the rice mutant lines induced by sodium azide. *Korean J. Breed. Sci.* 43: 23–31.

Siddiqui S, Meghvansi MK, Hasan Z (2007). Cytogenetic changes induced by sodium azide (NaN_3) on *Trigonella foenum-graecum* L. seeds. *S. Afr. J. Bot.* 73(4): 632–635. https://doi.org/10.1016/j.sajb.2007.06.005.

Srivastava P, Marker S, Pandey P, Tiwari DK (2011). Mutagenic effects of sodium azide on the growth and yield characteristics in wheat (*Triticum aestivum* L. em. Thell.). *Asian J. Plant Sci.* 10(3): 190–201. https://doi.org/10.3923/ajps.2011.190.201.

Sutardi S, Apriyana Y, Rejekiningrum P, Alifia AD, Ramadhani F, Darwis V, Setyowati N, Setyono DED, Gunawan G, Malik A, Abdullah S, Muslimin M, Wibawa W, Triastono J, Yusuf Y, Arianti FD, Fadwiwati AY (2023). The transformation of rice crop technology in Indonesia: Innovation and sustainable food security. *Agronomy* 13: 1. https://doi.org/10.3390/agronomy13010001.

Türkoğlu A, Tosun M, Haliloğlu K (2022). Mutagenic effects of sodium azide on in vitro mutagenesis, polymorphism and genomic instability in wheat (*Triticum aestivum* L.). *Mol. Biol. Rep.* 49: 10165–10174. https://doi.org/10.1007/s11033-022-07896-y.

Waheed A, Zhui L, Wang M, Hailiang X, Tong Z, Wang C, Aili A (2024). Integrative mechanisms of plant-salt tolerance: Biological pathways, phytohormonal regulation, and technological innovations. *Plant Stress* 14: 100652. https://doi.org/10.1016/j.stress.2024.100652.

Weldemichael MY, Baryatsion YT, Sbhatu DB, Abraha GG, Juhar HM, Kassa AB, Sibhatu FB, Gebremedhn HM, Gebrelibanos TS, Mossa

MM, Gebru MM, Meresa BK, Teklay M, Berhe BD, Gebru HA (2021). Effect of sodium azide on quantitative and qualitative stem traits in the M2 generation of Ethiopian sesame (*Sesamum indicum* L.) genotypes. *Sci. World J.* 2021: Article ID 6660711. https://doi.org/10.1155/2021/6660711.

Wu Z, Liu S, An B, Zhang H, Wu J, Li C, Long Y (2024). Mutagenesis and flowering promotion through sodium azide in vitro culture of *Cymbidium faberi* Rolfe. *Horticulturae* 10(8): 889. https://doi.org/10.3390/horticulturae10080889.