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ASSESSMENT OF ALUMINUM STRESS-TOLERANT RICE LINES DERIVED THROUGH MUTATION BREEDING

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SUMMARY

Mutation breeding is a promising technique used for improving crop plants' performance, including tolerance to aluminum in rice (*Oryza sativa* L.) cultivars. The presented research pursued developing aluminum-tolerant rice lines through mutation in two local rice cultivars, 'Mayas' and 'Adan'. Mutation induction using six doses of gamma irradiation included 50, 100, 150, 200, 250, and 300 Gy. The evaluation of root tolerance index proceeded for early selection of aluminum tolerant lines. In addition, root swelling, aluminum absorption, cross-sectional histology, and root lipid peroxidation incurred scrutiny. The results showed gamma irradiation (100 Gy) could produce aluminum stress tolerant lines from the cultivar Mayas. Aluminum-tolerant lines obtained totaled 91 through gamma irradiation in the local rice genotypes. The morphological traits of these aluminum-tolerant mutant lines underwent assessment for root elongation under stress conditions, root swelling occurrence, aluminum accumulation only at the root tip, cross-sectional histology with sclerenchyma thickening due to organic acids, and minimal cell wall damage. These lines need further evaluation to confirm their tolerance to aluminum stress, for rice cultivation on acid soils.

Keywords: Rice (*O. sativa* L.), mutation breeding, aluminum-tolerant lines, local rice genotypes, root tolerance index, cell wall damage

Key findings: Gamma irradiation (100 Gy) proved effective in producing aluminum-tolerant rice (*O. sativa* L.) lines. However, the higher doses of gamma irradiation negatively affected the performance of rice mutant lines, leading to disrupted plant growth and reduced aluminum tolerance.

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple for more than half of the world's population, cultivated in above 100 countries, with 90% of global production obtained from Asia (Fukagawa and Ziska, 2019). The cereal seeds are crucial components, rich in nutrients and of significant economic value for both humans and livestock (Akos *et al*., 2019). Rice is also vital in food security with a consistent rising demand of the world's booming population (Mohidem *et al*., 2022).

Various environmental factors, particularly acidic soils, constrain efforts to increase rice production. Approximately 40% of the world's arable land consists of acidic soils (Yadav *et al*., 2020). Acidic soil is a major challenge for rice cultivation in East Kalimantan, Indonesia, where intensive weathering and leaching processes in humid tropical climates lead to high aluminum saturation in the soil (Mulyadi, 2022). This metal is toxic for crop plants, posing a significant threat to sustainable agriculture development.

Aluminum (Al) is the third most abundant and toxic element in acidic soils (Bian *et al*., 2013). Under acidic conditions (pH < 5.0), Al dissolves into trivalent ions (Al^{3+}), directly affecting crop plants by inhibiting root elongation and disrupting nutrients uptake (Liu *et al*., 2016; Wei *et al*., 2021). The Al toxicity damages root tips and ultimately impacts rice production and grain quality (Li *et al*., 2014). By exposing the crop plants to Al poisoning, the symptoms—impaired root growth, root tip damage, and nutrients inhibition—can occur (Silva *et al*., 2012).

Efforts to increase soil pH by liming to reduce Al toxicity have proven less effective (Jiang *et al*., 2018). This method also demands considerable energy, costs, and time. Therefore, a better strategy is necessary to achieve sustainable goals of agriculture on acidic soils (Awasthi *et al*., 2017). Enhanced tolerance in rice plants to Al poisoning can be successful through utilizing local rice cultivars, which serve as source of genes controlling important tolerance traits in rice plants (Rembang *et al.,* 2018).

Local genetic resources of crop plants are valuable sources of genes managing essential traits that benefit breeding programs, including rice (Nurhasanah *et al*., 2018). The wide geographic distribution and long cultivation history of local rice contribute to its large genetic diversity and distinct characteristics. The adaptation of local rice to various regions and agroecological conditions can produce new alleles, potentially improving its quality (Roy *et al*., 2022).

Local rice genotypes are also familiar for its adaptability to both biotic and abiotic environmental stress factors, with the added advantage of its delicious taste. Local rice cultivars with high quality and excellent taste include 'Mayas' from Kutai Kertanegara and 'Adan' from Nunukan. However, pursuing studies on aluminum stress tolerance for these two rice cultivars have yet to occur. Therefore, it is imperative to enhance the aluminum stress tolerance in rice cultivars 'Mayas' and 'Adan' to achieve optimal results while preserving the positive characteristics of these local genotypes.

Efforts to improve various characteristics of local rice can proceed through several plant breeding programs, including mutation breeding (Kumar *et al*., 2021). Mutation breeding is highly effective in modifying traits to improve performance of cultivars, such as, plant height, flowering time, male sterility, grain quality, yield, and tolerance to abiotic and biotic stresses (Sobrizal, 2016). Mutagenesis has proven to be a powerful tool for enhancing and modifying the widely used rice genotypes, aligning them with the requirements of modern agriculture and commercial demands, thereby, contributing to superior cultivars' development (Andrew-Peter-Leon *et al*., 2021).

Studies on mutation by using gamma rays have shown to increase abiotic stress tolerance in crop plants at low doses (Katiyar *et al*., 2022). Gamma ray radiation has been effective to induce considerable variations in aluminum tolerance traits in the cultivars IR64 and Hawara Bunar, based on selection for relative root growth (RRG) (Rahayu, 2009). This indicates gamma ray-induced mutation has the potential to confer genetic changes for enhanced tolerance to abiotic stresses in rice, particularly aluminum tolerance. The promising study aimed to generate aluminum-tolerant lines through mutation breeding by subjecting the local rice cultivars 'Mayas' and 'Adan' to gamma ray irradiation and enhancing their aluminum tolerance while preserving the desirable characteristics of these local cultivars.

MATERIALS AND METHODS

Plant material

The plant materials used in this study were two local rice cultivars of 'Mayas' and 'Adan'. 'Mayas,' a well-known local rice cultivar from Kutai Kartanegara Regency, East Kalimantan, is distinct for its high quality and excellent taste (Nurhasanah *et al*., 2016). With these attributes, it is considerably a favored local rice cultivar in East Kalimantan. Additionally, 'Adan' rice cultivar from Nunukan Regency, North Kalimantan, is a highly esteemed local variety, valued for its superior quality, distinct characteristics, and exquisite taste. Its classification as a premium local rice often commanded higher prices due to its exceptional qualities (Rizal and Tarmisol, 2015). Besides these two local rice genotypes, two well-known national varieties, 'Mekongga' and 'IR64,' widely cultivated by Indonesian farmers, served as control genotypes in this study.

Gamma ray irradiation

Seeds of local rice cultivars 'Mayas' and 'Adan' sustained irradiation using gamma rays in the form of ^{60}Co, with irradiation doses of 50, 100, 150, 200, 250, and 300 Gy (Zou *et al*., 2023; Elsherbiny *et al*., 2024). Each treatment received seed allocation amounting to 100 grams. Although previous studies have shown lower doses are sufficient, using higher irradiation doses to induce more mutations that are significant could produce aluminum-tolerant plants, resulting in a broader and more diverse spectrum of mutations. For comparison, the wild types (non-irradiated seeds) of rice cultivars 'Mayas' and 'Adan' and the national superior rice cultivars, Mekongga as an aluminum tolerant (Sari *et al*., 2013), and IR64 as a susceptible genotype were samples in this study (Mackill and Khush, 2018).

Seeds germination

A hundred rice seeds of each irradiation dose, as well as, the wild type of 'Adan' and 'Mayas', and the control varieties Mekongga and IR64 grew on germination paper dampened with water. The paper, folded and placed in the dark, had a room temperature and remained for seven days. The calculation of percentage of germinated seeds with normal and abnormal sprouts helped evaluate the germination ability of the mutant seeds.

Aluminum stress treatment

Two aluminum stress treatment of 15 and 50 ppm $AlCl_3$ at pH 4.00 were functional in this experiment. The normal growth condition continued as a control treatment without applying aluminum in the growth solution at pH 5.8. Thirty sprouts with good growth and uniform root length were choices for each irradiation dose, serving each aluminum stress treatment. The sprouts of the wild type 'Mayas' and 'Adan', as well as, 'Mekongga' and 'IR64' were also specimens in this experiment as control genotypes in this study.

Before stress treatment, the selected sprouts' growth happened in 0.5 mM $CaCl_2$ solution for three days at a pH of 5.8, placing them on the floating net to prevent sprouts from drowning. Then, measuring the root length became the initial stage. Afterward, applying aluminum stress treatment to sprouts continued by adding 15 and 50 ppm of $AlCl_3$ to the $CaCl_2$ solution, decreasing the pH solution and arranging at pH 4.00 by adding HCl 0.1 N. The aluminum stress experiment proceeded for 24 hours. The root length's measurement helped calculate their Root Tolerance Index (RTI).

Root tolerance index (RTI)

The maximum root length of the sprout experiencing Al stress divided by the maximum root length of the normal growth condition (control – without aluminum treatment) after 24 hours in the Al stress treatment helped determine the root tolerance index (RTI) (Bhattacharjee *et al*., 2023). Based on the RTI values, the mutant lines and the control genotypes categorization comprised tolerant (RTI > 0.9), moderate (0.7 ≤ RTI ≤ 0.9), and sensitive (RTI < 0.7).

Root swelling

During aluminum stress treatment, observing root swelling occurred. Root swelling visually ensued after exposing the sprouts to aluminum treatment for three hours. Sprouts with aluminum-resistant roots showed little to no swelling, whereas in aluminum-sensitive plants, root swelling was prominent. This swelling is an indicator of the plant's defense mechanism under aluminum stress (Liu *et al*., 2016). Recording the occurrence of root swelling continued, with selected samples further examined under a microscope.

Aluminum accumulation

After 24 h of exposure to $AlCl_3$ in the Al stress treatment, the root samples, cut into 1–2 cm segments, bore gentle washing in 50 mL of distilled water for 15 min. The transfer of samples into a 0.2% Mayer's hematoxylin solution (0.02%$NaIO_3$) reached 30 min of soaking for staining the roots. Following staining, roots' rinsing with distilled water took 15-22 min before air-drying. The aluminum accumulation in the roots underwent a stereoscopic microscope observation. Qualitative assessment of aluminum absorption ensued based on the intensity of the color after staining; a more intense color indicated higher aluminum absorption (Awasthi *et al*., 2017).

Histological observation

The stained root samples with hematoxylin attained fixing with a 4% FAA (formalin-acetic acid-alcohol) solution for 24 h. After fixation, the root apices received distilled water washing. Cross-sections, approximately 3.00 mm from the root tip, incurred manual cutting as thinly as possible using a razor blade. The sections' scrutiny under a light microscope had a 10×10 magnification, with images captured using an Optilab camera.

Analysis of lipid peroxidation in root cell membranes

Lipid peroxidation observation can assess the extent of cell membrane damage caused by aluminum toxicity. This damage detection can be qualitatively by observing the red coloration in the roots when staining the roots with 5% Schiff reagent for 20 min, detecting aldehydes derived from lipid peroxidation. Following staining, rinsing the roots used a sulfite solution (0.5% [w/v] $K_2S_2O_5$ in 0.05 M HCl) for 10 min. The stained roots remained in the sulfite solution to preserve the color. The red color was visible in the roots under the microscope for lipid peroxidation (Awasthi *et al*., 2017; Jaiswal *et al*., 2018).

RESULTS AND DISCUSSION

Mutation breeding plays a crucial role in improving plant traits, such as, tolerance to abiotic and biotic stresses, making it a key strategy in addressing inevitable challenges in food production. Mutation breeding refers to the use of artificial mutagenesis to develop new biological cultivars. One approach is radiation mutagenesis, which induces further complex genetic mutations and yields more advantageous mutant phenotypes (Ma *et al*., 2021). Radiation-induced mutation breeding offers unparalleled advantages, characterized by a broad mutation spectrum and high mutation efficiency (Shirasawa *et al*., 2016). Thus, irradiation, particularly with gamma rays, can promote structural and metabolic changes in plants.

Germination rate of mutant lines

An increase rate of abnormal sprouts in the seven-day-old sprouts due to increased irradiation dose applied to the seeds of the rice cultivars 'Mayas' and 'Adan' (Figure 1). Increasing gamma irradiation doses significantly affects the germination ability of rice mutant lines. An increased percentage of abnormal sprouts was visible due to higher irradiation doses. With elevated irradiation doses, similar results appeared in crop plants at a dose of 250 Gy (Costa *et al*., 2022). Low-dose irradiation treatment causes phenotypic variations in plants without lethal effects. Zou *et al*. (2023) also reported gamma irradiation positively influenced rice seed vigor at doses of 50 and 100 Gy. However, it had a negative impact with higher doses. Irradiation with 100 Gy positively modified the seed vigor. Still, beyond this dose, the seed vigor lessened. Additionally, low doses of gamma irradiation can stimulate positive effects, such as, raising and speeding up germination percentage and variations in root and shoot length. These effects resulted in variations at the morphological, physiological, biochemical, metabolic, and molecular levels (Villegas *et al*., 2023).

The higher the dose of irradiation, the shorter the sprouts obtained in mutant lines, as observed in mutant sprouts of the rice cultivars 'Mayas' and 'Adan'. A marked difference was evident between the positive effects of the lowest dose (50 Gy) and the negative effects of the highest dose (300 Gy). The seeds exposed to gamma ray doses, ranging from 200 to 300 Gy, resulted in the development of stunted and abnormal sprouts. In these instances, the root length typically reached a few millimeters and even less (Figure 2). The genetic and physiological damage to embryo cells due to mutagenesis also alter the seed germination, cell death, abnormal cell division, tissues and organ damage, and eventually reduced plant growth (Lagoda, 2012; Ma *et al*., 2023).

Variations in germination morphology, such as, reduced height and shorter sprout roots, can be due to increased doses of gamma radiation, which disrupt the cell division process from DNA mutation (Naibaho *et al*., 2021). This disruption proved associated with the cell cycle during somatic cell division and various damages throughout the genome (Preuss and Britt, 2003). A series of stress responses induced by gamma radiation, including the reactive oxygen species (ROS) scavenging system and transcription factors, inhibit the upregulation of ribosomal protein gene expression, thereby, hindering cell division. This cell division inhibition serves as a primary cause of germination suppression, leading to hormonal imbalances and

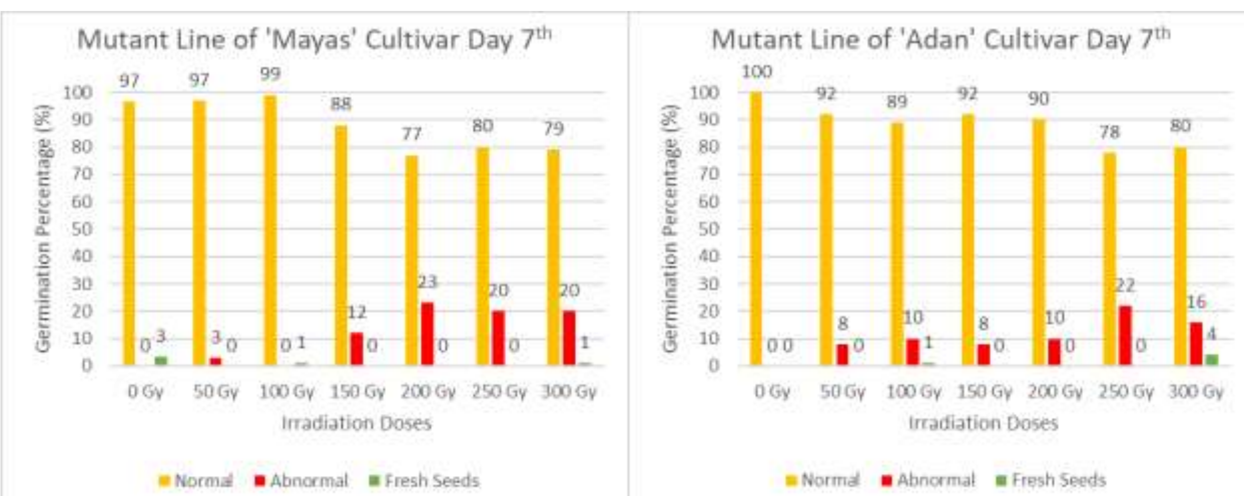

Figure 1. Germination of mutant line seeds of rice cultivars Mayas and Adan on the 7th day.

Figure 2. The sprouts of mutant lines of the rice cultivars Mayas (k_1) and Adan (k_2) at different radiation doses. g_1 = 50 Gy, g_2 = 100 Gy, g_3 = 150 Gy, g_4 = 200 Gy, g_5 = 250 Gy, and g_6 = 300 Gy.

withholding the upregulation of stored lipid breakdown genes (Liu *et al*., 2021). Processes, such as, the disruption of auxin synthesis, variations in ascorbic acid content, and various physiological and biochemical disorders can inhibit the germination and plant development due to mutation induced by gamma radiation (Shah *et al*., 2008; Kiong *et al*., 2008).

Root tolerance index

Based on the root tolerance index (RTI) values, assessing the level of aluminum tolerance is feasible in the control genotypes and the wild types. Rice cultivars 'Adan' and 'Mayas' received a moderately tolerant category, while rice genotypes 'IR 64' and 'Mekongga' emerged sensitive to aluminum (Table 1). Aluminum-tolerant lines came only from cultivar 'Mayas' irradiated with gamma rays at the doses ranging from 50 to 200 Gy (Figure 3). No tolerant lines surfaced with higher irradiation doses of 250 and 300 Gy. However, the highest percentage of aluminum-tolerant mutant lines was attainable from the cultivar 'Mayas' at a low irradiation dose (100 Gy). Conversely, in the mutant lines derived from cultivar 'Adan', no aluminum-tolerant lines were evident. A low irradiation dose of 50 Gy produced the utmost number of moderately aluminum-tolerant genotypes in this population. Increasing the irradiation dose reduced the percentage of tolerant or moderately tolerant genotypes in mutant lines derived from both cultivars 'Mayas' and 'Adan' (Table 2).

The wild types of the rice cultivars 'Adan' and 'Mayas' garnered a moderately tolerant classification to aluminum stress based on their RTI values, which were higher than 0.7. It indicated aluminum stress causes less than a 30% disruption in root growth compared with the control treatment. Nurhasanah *et al*. (2019) reported rice cultivar 'Mayas' was also an identified iron-tolerant cultivar. Tripathi *et al*. (2018) also suggested tolerance to iron toxicity could help alleviate the stresses caused by other metal ions in crop plants, with the said hypothesis also supported by the present findings. In addition, the aluminum-sensitive control genotype, 'IR64,' bore further confirmation as sensitive based on

Table 1. Root tolerance index (RTI) of the control genotypes and wild types of the rice cultivars Mayas and Adan.

Genotype	RTI $AlCl_3$ 15 ppm	RTI $AlCl_3$ 50 ppm	RTI Average	Category
'Mayas' wild type	0.68	0.77	0.73	Moderate
'Adan' wild type	0.79	0.79	0.79	Moderate
Mekongga	0.67	0.66	0.66	Sensitive
IR64	0.59	0.63	0.61	Sensitive

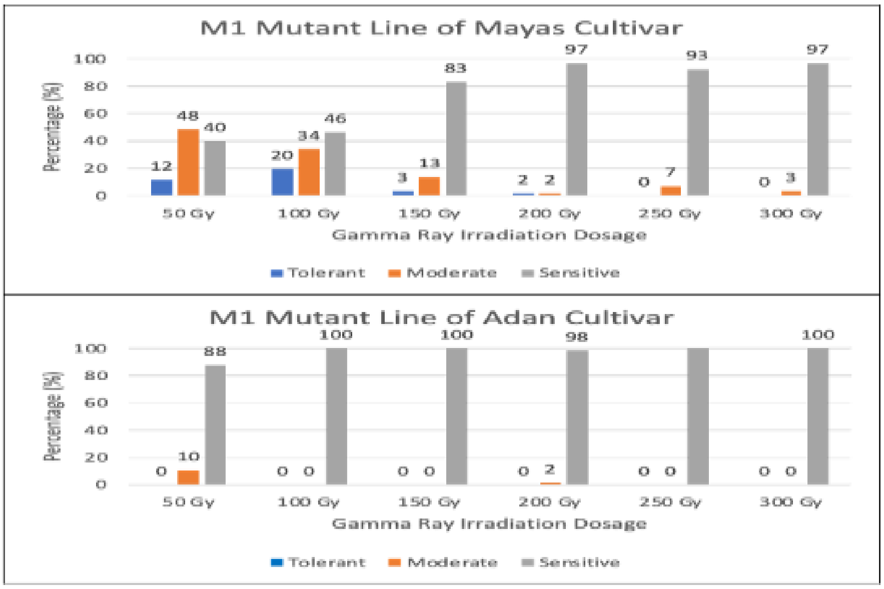

Figure 3. Percentage of tolerant, moderate, and susceptible rice mutant lines against aluminum stress based on RTI values.

Table 2. Number of tolerant, moderate, and sensitive rice mutant lines exposed to 15 and 50 ppm of $AlCl_3$ based on RTI values.

Irradiation Dose	Tolerant		Moderate		Sensitive	
	15 ppm	50 ppm	15 ppm	50 ppm	15 ppm	50 ppm
Mutant derived from 'Mayas' cultivar						
50 Gy	3	4	14	15	13	11
100 Gy	6	5	14	5	8	18
150 Gy	2	0	4	4	24	26
200 Gy	1	0	1	0	28	30
250 Gy	0	0	1	3	29	22
300 Gy	0	0	0	2	30	28
Total	12	9	34	29	132	135
Mutant derived from 'Adan' cultivar						
50 Gy	0	0	1	5	29	22
100 Gy	0	0	0	0	30	29
150 Gy	0	0	0	0	30	30
200 Gy	0	0	1	0	29	30
250 Gy	0	0	0	0	30	30
300 Gy	0	0	0	0	30	28
Total	0	0	2	5	178	169

the RTI value under 15 and 50-ppm AlCl3 stress treatments. An earlier research has evaluated cultivar 'IR64' as sensitive to aluminum, not only through morphological assessments, but also on molecular analysis (Mackill and Khush, 2018). The rice cultivar 'Mekongga,' previously identified as a tolerant genotype based on morphological observations under 15 ppm aluminum stress using Root Re-Growth (RRG) measurements (Sari *et al*., 2013), revealed sensitive according to the RTI value at the 15 and 50 ppm $AlCl_3$ stress treatments (Table 1). This discrepancy highlights that relying solely on morphological observations can lead to biased conclusions. Therefore, comprehensive evaluation based on the morphological and molecular analyses proved crucial for accurately assessing aluminum tolerance.

Based on the RTI values of the mutant rice seedlings, the early selection for aluminum tolerance derived from the cultivars 'Mayas' and 'Adan' signified as feasible. Aluminum-tolerant rice lines were notable more frequently among the mutants derived from cultivar 'Mayas' than 'Adan'. None of the mutant lines derived from the cultivar 'Adan' reached a category as aluminum tolerant (Figure 3). However, the wild types of the cultivars 'Adan' and 'Mayas' gained the level as moderately tolerant to aluminum (Table 2). Previous studies also revealed a reduction in plant performance associated with higher doses of gamma irradiation (Naibaho *et al*., 2021; Solim and Rahayu, 2021).

With aluminum stress, in sensitive rice lines, the root growth inhibition prevailed, and roots become shorter due to thickening. Aluminum poisoning initially inhibited the root growth by decreasing cell division and elongation at the root tip (Yang and Horst, 2015), leading to root cap shortening (Jaskowiak *et al*., 2019). Short-term exposure to low doses of aluminum inhibits the root growth, while long-term exposure to higher aluminum concentrations showed no inhibitory effect (Zhou *et al*., 2011). This indicates different rice cultivars have different response mechanisms to aluminum toxicity. Bhattacharjee *et al*. (2023) found for susceptible genotypes, the RTI values indicated cytotoxic aluminum concentrations, whereas tolerant genotypes could stimulate the root growth.

Root swelling

In aluminum-tolerant sprouts, the roots tend not to swell, whereas, in the sensitive genotypes, root swelling was prominent (Figure 4). Root swelling, as observable under a microscope, is an indicator of the plant's defense mechanism against aluminum stress (Liu *et al.,* 2016). In addition to root swelling, alterations in root structure, such as the formation of cracks, also occurred, following exposure to aluminum stress (Figure 5).

Aluminum treatment induced swelling in the roots of sensitive mutant rice lines. This root swelling may refer to increased ethylene production (Liu *et al*., 2016). The symptoms of root swelling suggested the cytoskeleton might be a target of aluminum toxicity, as aluminum stress causes variations in root tissue structure and biochemical processes (Blancaflor *et al*., 1998). This phenomenon was apparent in the mutant sprouts, which exhibited cracks in the roots (Figure 5). Swollen and distorted root tip cells indicate abnormal changes in cell shape and structure due to aluminum pressure, and it was a belief to be indicators of aluminum sensitivity. Typically, after 24 h, root swelling was no longer visible because of the peeling off in the epidermis and outer cortex parts, signifying aluminum increases the stability of microtubules in the swollen cells. The stabilizing effect of aluminum in the outer cortex coincides with the growth inhibitory effects (Blancaflor *et al*., 1998).

Aluminum stress decreases the diameter of stele, while the width of the cortex increases due to cell enlargement, causing swelling at the root tips in alfalfa (Wang *et al*., 2016). This cell enlargement resulted from Al^{3+} induced vacuole enlargement. Vacuole enlargement may be necessary for plants to store excess Al^{3+} and adapt to Al^{3+} stress conditions (Kochian *et al*., 2015). However, this variation can disrupt the root functions, such as, nutrient absorption and reduce root

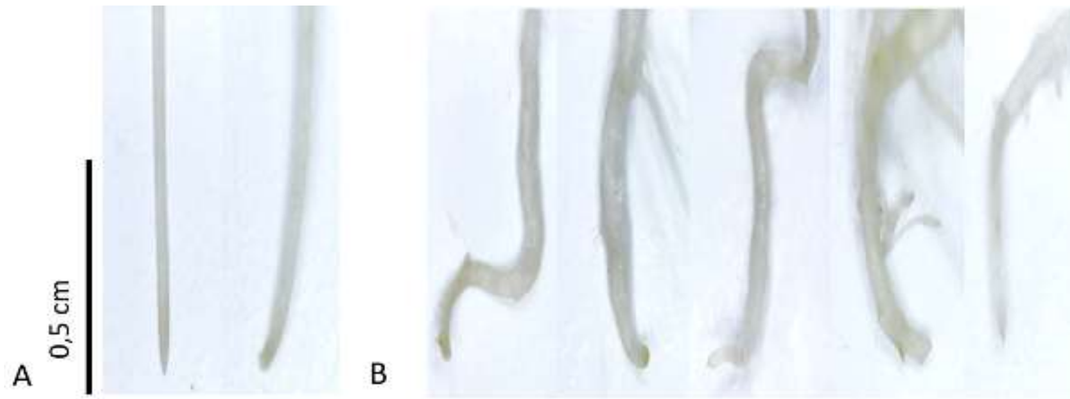

Figure 4. Roots swelling of rice line sprouts. A) Without $AlCl_3$ treatment (normal growth condition), B) $AlCl_3$ stress treatment.

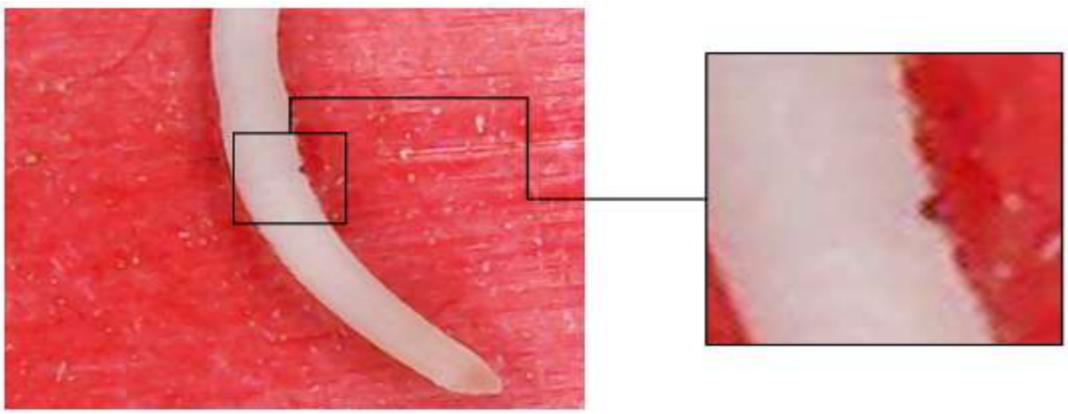

Figure 5. Response to variations in root structure in the form of root cracks after aluminum stress treatment.

growth, and, thus, the vacuole enlargement may be a significant characteristic of Al^{3+} toxicity in crop plants (Wang *et al*., 2016).

Aluminum absorption

Hematoxylin staining showed absorption of aluminum in the roots (Figure 6). In the tolerant rice lines, aluminum tends to concentrate only at the root tips. In contrast, in sensitive rice lines, the color concentration was evident throughout the root zone, with the most intense staining at the root tips and secondary roots.

Hematoxylin is an aluminum indicator dye useful for assessing the localization and accumulation of aluminum in root tips (Awasthi *et al*., 2017). Higher aluminum concentrations in root tissue result in more intense and darker coloration. In the roots of rice cultivar 'IR64,' dark-colored spots were notable, which were invisible in the other control genotypes. Additionally, the sprouts exhibited an increase in root diameter by exposing to aluminum concentration of 50 ppm (Figure 6). The binding of hematin (oxidized hematoxylin and bluish purple color) indicates aluminum localization in the cytoplasm (Awasthi *et al*., 2017). Hematoxylin staining showed aluminum can easily enter the root cells of rice sprouts within 24 h, leading to aluminum accumulation at the root tip in tolerant, moderate, and sensitive mutant rice sprouts. Tolerant sprouts showed less accumulation at the root tip, while

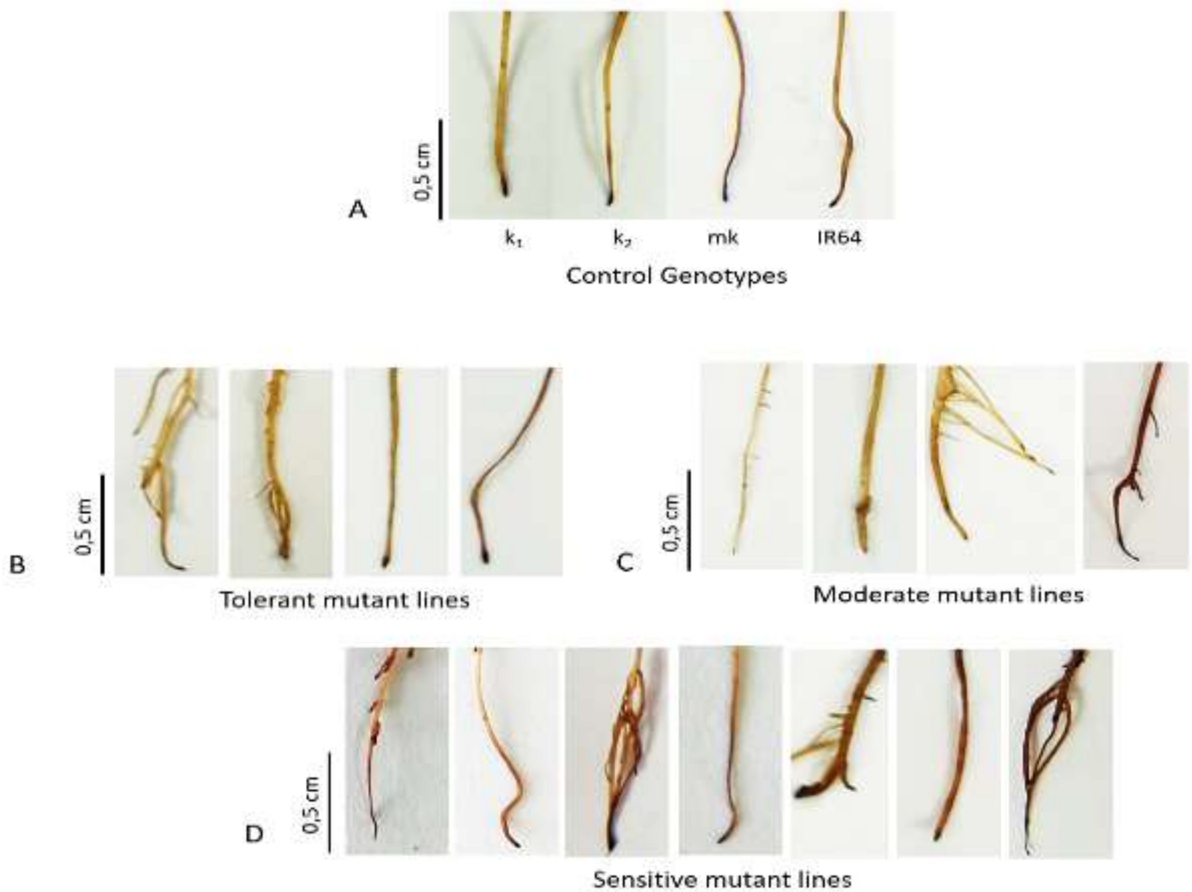

Figure 6. Hematoxylin staining of the rice control genotypes (A) and mutant lines (B, C, D). Wild type of cultivars Mayas (k1) and Adan (k2), and Mekongga (mk) and IR 64.

susceptible rice sprouts exhibited a higher aluminum accumulation and, with time, became concentrated and deeper. Similarly, Bhattacharjee *et al*. (2023) explained rice genotypes sensitive to aluminum toxicity displayed a dark blue color, indicating higher aluminum accumulation in the roots, while tolerant rice exhibited negligible coloring, indicating the aluminum toxicity-tolerant genotypes accumulated less aluminum in their roots.

Histological observations

The roots of mutant lines treated with aluminum stress exhibited differences in the cell walls, epidermis, and cortex (Figure 7). In tolerant rice lines, the cell walls thickened, root hair formation frequently occurred in the epidermis, and an enlargement in diameter occurred. Additionally, the epidermis thickened with a normal stele, and variations in root shape were visible, while growth continued. Sensitive roots displayed various responses, such as, an increase in diameter with thickening of the fibrous part of the epidermis, variations in epidermis shape, and variability in root hairs based on the stiffness of the epidermal cells. Stiffness also emerged in the epidermis cells and other parts.

The cross-sections of roots in rice mutant lines exhibited various responses to aluminum stress, which altered the shape of the root cross-section (Figure 7). Tolerant mutant lines showed specific variations, such as, thickening of the sclerenchyma with a dense cortex, thickening of the sclerenchyma and growth of root hairs with a loose cortex.

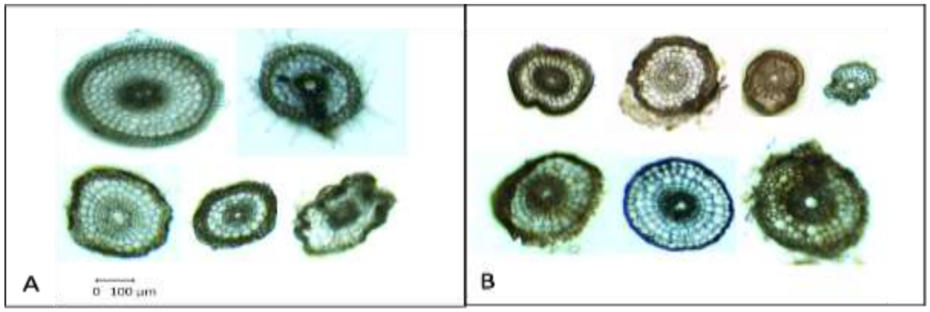

Figure 7. Histology of rice cross-sectional roots. A) Control genotypes at normal growth, B) Control genotypes at aluminum stress treatment, C) Tolerant mutant lines, and D) Rice sensitive mutant lines.

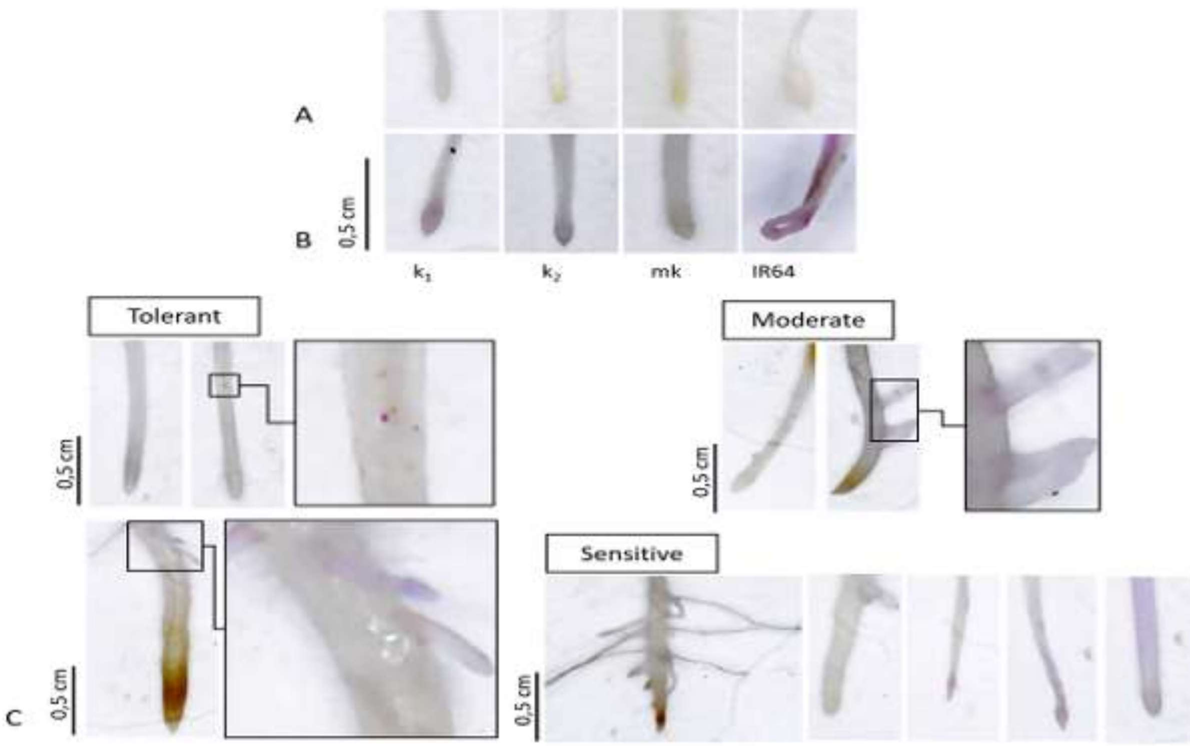

Figure 8. Root lipid peroxidation. A) Control genotypes at normal growth, B) Control genotypes at aluminum stress treatment, C) Tolerant, moderate and sensitive rice mutant lines at aluminum stress treatment.

Moreover, thickening of the epidermis and stele, variations in the shape of root surface, stele, and visible stretching of the cortex showed, while still maintaining root growth. Moderate genotype roots were unidentifiable in the cross-section analysis, as sampling continued randomly by observing different root conditions. In sensitive genotype sprouts, the variations included thickening of the epidermis and stele, as well as, other specific responses to aluminum stress. The control treatment of the sensitive rice cultivar 'IR64' exhibited significant cell membrane damage with dense staining in the roots (Figure 8). This aligns with the explanation in the root swelling section, where root swelling was no longer visible due to the peeling off from the epidermis and outer cortex. Additionally, aluminum-induced formation of reactive oxygen species (ROS) leads to lipid peroxidation, which is a prominent symptom of oxidative stress. This process accelerates membrane loss and protein degradation, ultimately resulting in programmed cell death (Ofoe *et al*., 2023). In the rice cultivar 'Mayas,' there was a faint and very thin coloration at the root tips, while the cultivar 'Adan' displayed slight redness throughout the roots (Figure 8). The more intense the color, the higher was the level of lipid peroxidation, indicating increased sensitivity to aluminum stress.

Lipid peroxidation in root cell membranes

Root cell membrane damage was evident through the red color visible in the roots under the microscope. In tolerant rice lines, the root tips generally appear robust with no signs of damage, although occasional faint staining from Schiff's reagent was notable in secondary roots and specific locations, indicating the impact of aluminum stress. In moderate rice lines, a slight red color appeared in secondary roots; however, the primary roots occurred unaffected. Conversely, sensitive rice cultivars displayed significant signs of damage, including coloring in the secondary roots, root shortening with color absence at the tips, colored root tips with slender roots, and increased cell damage extending to the deepest parts of the roots (Figure 8).

Sensitive rice mutant lines showed differences in root tip morphology, with blunter root tips than tolerant and moderate rice mutants. The roots of tolerant and sensitive rice sprouts appeared more robust after staining. Lipid peroxidation remains a critical outcome of aluminum exposure in tolerant, moderate, and sensitive rice plants. The most noticeable lipid peroxidation occurs in sensitive sprouts, characterized by distinctive coloring at the root tip and throughout the roots. The observed staining on the root surface indicates lipid peroxidation in root cells was directly from aluminum interaction on the root surface (Awasthi *et al*., 2017; Fendiyanto *et al*., 2019; Manzoor *et al*., 2024).

CONCLUSIONS

The gamma irradiation dose of 100 Gy revealed effective in inducing mutation and increasing the level of tolerance to aluminum stress in rice mutant lines derived from the cultivar 'Mayas'. Aluminum-tolerant mutant lines exhibited root elongation under stress conditions, no root swelling, aluminum accumulation at the root tips, and continuous root elongation despite variations in root surface structure. Additionally, these rice lines displayed excellent histological cross-section conditions, with thickening in the sclerenchyma and slight thickening in the epidermis, and they experienced less cell membrane damage compared with the sensitive rice genotypes. Conversely, higher doses of gamma irradiation negatively influenced the performance of rice mutant lines, leading to disrupted plant growth and reduced tolerance to aluminum stress, shifting from moderate to sensitive levels. Increased irradiation doses resulted in more sensitive rice lines to aluminum stress.

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