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# Low-Dose γ-Irradiation Priming of Seeds Alleviates Aluminum-Induced Oxidative Stress and Genotoxicity in the Oil Seed Crop, Niger, *Guizotia abyssinica* (L.f.) Cass

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**ABSTRACT |** Aluminum ($Al^{3+}$) stress is a primary factor limiting crop productivity in the acidic soils of the tropical and sub-tropical regions of the world. Seed priming is a viable approach to sustain crop productivity by counteracting environmental stress. In the present study, we tested the efficacy of seed priming with low dose of γ-irradiation (0.01 kGy) in the oil seed crop, Niger, *Guizotia abyssinica* (L.f.) Cass. as the model plant against the challenging treatment of $Al^{3+}$ (10 mM) at pH 4.5. Replicated experiments in four sets with appropriate negative and positive controls were conducted. Indices of phytotoxicity (seedling growth and cell death), oxidative stress (generation reactive oxygen species, ROS and lipid peroxidation), genotoxicity (mitotic index and frequency, chromosomal aberrations, and micronucleus formation) and the activities of antioxidant enzymes (catalase, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase) in the leaf tissue and root meristems of the germinating seeds were determined. Priming of seeds with γ-irradiation caused significant inhibition of seedling growth marked by mild oxidative stress with little or no genotoxicity. Seeds treated with $Al^{3+}$ alone revealed significant inhibition of seedling growth marked by significant induction oxidative stress and genotoxicity. However, seeds which were consecutively primed with γ-irradiation and exposed to $Al^{3+}$ challenge, exhibited amelioration of oxidative stress and genotoxicity. Overall, the results of the current study demonstrated that γ-irradiation priming of the dry seeds of *G. abyssinica* invoked an adaptive response through eliciting mild oxidative stress that ameliorated plant tolerance to $Al^{3+}$ toxicity.

**KEYWORDS |** Aluminum phytotoxicity; Adaptive response; Genotoxicity; Ionizing radiation; Oil seed; Oxidative stress; Seed priming

**ABBREVIATIONS |** APX, ascorbate peroxidase; CAT, catalase; EDTA, ethylenediaminetetraacetic acid; GPX, guaiacol peroxidase; GR, glutathione reductase; MDA, malondialdehyde; NADH, nicotinamide adenine dinucleotide hydrogen; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid

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**CONTENTS**



## 1. INTRODUCTION

Aluminum ($Al^{3+}$) is the third most abundant element in the Earth's crust next to oxygen and silicon, constituting nearly 8.8% by weight (88 g/kg) of the Earth's elemental load. The state of Odisha in India having 51% of the global bauxite deposits has been a major multinational hub for bauxite mining and Al-based industries [1]. Al belongs to the group IIIA of the periodic table and, owing to its lightness (specific gravity 2.7), has been the most sought after light metal in industries [2]. With the increasing global demand for Al, anthropogenic activities related to bauxite-mining, Al-refining, and smelting are steadily increasing. Al enters the environment naturally through the weathering of rocks and minerals. Anthropogenic release of Al into the environment in the form of air emissions, waste water effluents, and solid waste is primarily associated with the industrial processes [3].

$Al^{3+}$ phytotoxicity is a primary factor limiting crop productivity in the acidic soils, which comprise up to 40% of the total world's arable lands in the tropics and subtropics [4, 5]. Soil acidification resulting from either continuous use of ammonia- and amide-containing fertilizers or the nitrogen fixation by the legumes aggravates $Al^{3+}$ toxicity [6]. $Al^{3+}$, having strong binding affinity for proteins, inorganic phosphate, nucleotides, RNA, DNA, carboxylic acid, phospholipids, and anthocyanin, is considered highly reactive [7]. Al phytotoxicity is marked by lipid peroxidation [8], mitochondrial dysfunction [9], and apoplastic oxidative burst [10], which ultimately results in oxidative stress to plants [11, 12]. Al, being capable of inducing DNA damage and triggering genotoxic stress, shows an impact on the entire biota and/or biodiversity [13–15].

Priming is being considered an important approach for enhancing the plant defense against both biotic and abiotic stresses [16–19]. The process of priming (also referred to as 'hardening' or 'conditioning') involves prior exposure to an abiotic stress factor making a plant more resistant to future exposure of stress. This feature in higher plants indicates some capacity

for 'memory' or 'adaptive response' considered as a nonspecific phenomenon in which an exposure to minimal stress could result in the increased resistance to the exposed stress or other types of stress hours or days later [20]. It is defined as the pre-exposure of seeds or young seedlings to chemical agents or to abiotic stressors (such as radiation, salinity, drought, and cold, among others) making them more resistant to subsequent stresses through a mechanism of signal transduction [21]. In other words, alleviation has been observed following the exposure to two consecutive acute stresses. After priming the seeds first time with a low dose of radiation or abiotic stress, the effect observed during a second exposure to stress is reduced, which has been termed adaptive response with profound implications in stress resistance in crop plants (for review see [22]).

The phenomenon of radio-priming or radio-adaptive response has been observed in cell survival studies with yeast, bacteria, protozoa, algae, higher plants, insect cells, and mammalian and human cells in vitro, and in studies on normal animal tissue models in vivo with implications in radiation epidemiology, cosmic biology, and radiation therapy [23, 24]. From an agriculture perspective, seed-priming has been viewed as a short-term strategy that rescues crops from any possible adverse impact and sustains productivity despite stress (for reviews see [25–27]).

Genomic protection studies have been documented in *Allium cepa* root tip cells, showing two low and high consecutive X-ray irradiations have reduced the number of chromatid breaks during an adaptive response involving the error-free DNA repair system [28]. The γ-irradiated dry seeds (8 Gy) of *Oryza sativa* have exhibited a greater resistance to saline stress than the non-irradiated ones [29]. *Trifolium alexandrinum* plants grown from seeds primed with low-dose γ-irradiation (0.05 kGy) have exhibited enhanced induction of antioxidants (both enzymatic and non-enzymatic antioxidants) as compared to the same exposed to higher doses of γ-irradiation, suggesting their heightened ability of amelioration of ozone stress [30]. Having this as the premise, in the present study, we investigated the impact of seed-priming with low dose γ-irradiation on the Al-induced oxidative stress and genotoxicity in niger (*Guizotia abyssinica*) as the model system, which is an important oil seed crop being cultivated in this region of Southern Odisha.

## 2. MATERIALS AND METHODS

### 2.1. Procurement and Handling of Seeds

Seeds of *Guizotia abyssinica* (L.f.) Cass. (niger) were collected from the local farmers. Prior to experimentation, the seed-moisture content was equilibrated to ~20% following the standard procedure of drying and vacuum desiccation over glycerol-water mixture [31].

### 2.2. Test Chemical and Experimental Solution

The test chemical used in our present experiments is aluminum chloride ($AlCl_3$, HiMedia, India). Stock solution (500 mM) of $AlCl_3$ was prepared in water at pH 4.0. Test solutions of desired concentrations of $AlCl_3$ were prepared in sterile tap water by appropriate dilution and adjusting the pH to 4.5 ensuring that during treatments the metal ion was in the soluble form ($Al^{3+}$) and available for uptake by the seeds [32].

### 2.3. Seed Priming and Treatment

Dry seeds of *G. abyssinica* in polythene bags (5 × 8 cm) were irradiated at low doses of γ-rays ranging from 0 (control) to 0.1, 0.5, and 1.0 kGy emitted from a $^{60}$Co-source (Gamma Chamber-5000, BRIT, Mumbai, India; emitting a dose of ~0.133 kGy/min). Dry seeds following first priming with γ-irradiation (0.01–0.1 kGy) and then pre-soaking overnight in distilled water were treated with $Al^{3+}$ in test solutions at doses of 0, 10, 20, and 30 mM for 12 h. After thorough washing in running tap water, the treated seeds were then set for germination on moist papers in Petri dishes under aseptic condition in dark at 24 ± 1°C. Appropriate negative (distilled water) and positive (γ-irradiation and Al-treatment) controls were maintained and handled alike.

### 2.4. Germination and Seedling Growth

After recording the germination at 36 h of seed setting, root tips from at least 15 germinating seeds from each irradiation dose were excised, fixed in acetic acid:ethanol (1:3) for 24 h, and then stored in 70% ethanol at 4°C for cytological analysis. The remaining of the germinating seeds were allowed to grow as seedlings that were kept under cool fluores-

cent light at an intensity of 200 µmol $m^{-2}$ $s^{-1}$ with a 12/12 h light/dark cycle. Care was taken to maintain the seedlings at constant moisture by daily replenishment of equal volumes of water into the Petri plates to correct the loss of water by evaporation. On day six, seedling heights were recorded by measuring the lengths of the shoots from at least 75 randomly chosen seedlings from each treatment. The first leaves excised at the base were processed for biochemical analysis.

### 2.5. Determination of ROS, Cell Death, and Lipid Peroxidation in Seedling Leaf Tissue

Cellular generation of reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet-}$) [33], hydrogen peroxide ($H_2O_2$) [34], and hydroxyl radical ($OH^{\bullet}$) [35], was determined spectrophotometrically on a Double Beam UV-Visible Spectrophotometer (Model UV-3000+, Lab India Instruments, Mumabi, India). The procedures for determining the above ROS are described below.

#### *2.5.1. Determination of $O_2^{\bullet-}$ Generation*

For the determination of $O_2^{\bullet-}$, freshly weighed (2 g) leaf samples (6-day-old) were homogenized in 2 ml of 50 mM sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 12,000 × *g* for 15 min, and 500 µl of the supernatant was mixed with 3 ml reaction mixture containing 1 mM epinephrine and 1 mM nicotinamide adenine dinucleotide hydrogen (NADH, HiMedia, Mumbai, India) dissolved in 50 mM sodium phosphate buffer (pH 7.4). The level of $O_2^{\bullet-}$ produced was calculated by monitoring the rate of epinephrine oxidation to adrenochrome as determined from the absorbance difference ($A_{485}$–$A_{575}$, $\varepsilon = 2.96$ $mM^{-1}$ $cm^{-1}$) and expressed in µmol per gram of fresh weight (FW).

#### *2.5.2. Determination of $H_2O_2$ Generation*

For the determination of $H_2O_2$, approximately 2 g of leaf samples were homogenized at 4ºC in 4 ml of 0.2% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 × *g* for 15 min and 0.5 ml of the supernatant were mixed with 1 ml of 10 mM sodium phosphate buffer (pH 7.0) and 2 ml of 1 M potassium iodide. Levels of $H_2O_2$ in the supernatant were determined by measuring the absorbance of the assay mixture at 390 nm with appropriate standards and expressed in nmol per gram of FW.

#### *2.5.3. Determination of $OH^{\bullet}$ Generation*

For the determination of extent of the cellular $OH^{\bullet}$, leaf samples (1 g) were homogenized in 10 mM sodium phosphate buffer (pH 7.4) containing 15 mM 2-deoxy-D-ribose and then centrifuged at 12,000 × *g* for 15 min. The supernatants were then incubated at 37ºC for 2 h and aliquots of 0.2 ml of the supernatants were added to the reaction mixture containing 3 ml of 0.5% (w/v) thiobarbituric acid (TBA, 1% stock solution made in 5 mM NaOH) and 1 ml glacial acetic acid, heated at 100°C in a water bath for 30 min, and cooled down to 4ºC for 10 min before the measurement. The absorbance of malondialdehyde (MDA)/TBA-derived color product, as an index of 2-deoxy-D-ribose oxidation by $OH^{\bullet}$, was measured at 532 nm and the extent of 2-deoxy-D-ribose oxidation was calculated by using the molar extinction coefficient of MDA/TBA-derived color substance ($\varepsilon = 155$ $mM^{-1}$ $cm^{-1}$) and expressed in nmol MDA equivalent per gram of FW.

#### *2.5.4. Determination of Cell Death*

The cell death in leaf tissue was determined by the Evans blue staining method [36]. Freshly excised leaves were immersed in 0.25% (w/v) aqueous solution of Evans blue for 6 h and left overnight in the same solution. The leaves were then bleached in boiling ethanol (95% v/v) to remove chlorophyll from the leaves. For the quantification of cell death, stained leaves weighing ~150 mg from each dose were immersed in the dye-extraction solution consisting of 50% (v/v) ethanol in 1% (w/v) sodium dodecyl sulfate (SDS) for 1 h at 50°C. Absorbance of the Evans blue released into solution was determined spectrophotometrically at 600 nm.

#### *2.5.5. Determination of Lipid Peroxidation*

Lipid peroxidation was measured as the level of MDA formed from the ROS-catalyzed membrane polyunsaturated fatty acid peroxidation by using the TBA reaction [37]. Leaf samples (500 mg) from the unexposed control and irradiated seedlings were ho-

mogenized in 3 ml of 20% (w/v) TCA and centrifuged at 10,000 × *g* for 10 min. One ml of the supernatant was added to the reaction mixture containing 20% (w/v) TCA and 0.5% (w/v) TBA, followed by heating at 95ºC for 30 min. After stopping the reaction by cooling the test tubes on ice, the reaction mixture was then centrifuged at 10,000 × *g* for 15 min. The absorbance of the resulting supernatant was determined at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from that determined at 532 nm. The concentration of MDA was calculated by using the molar extinction coefficient ($\varepsilon$ = 155 $mM^{-1}$ $cm^{-1}$) of MDA-TBA complex and expressed as nmol per gram of FW.

### 2.6. Determination of Soluble Protein and Antioxidant Enzyme Activities

#### *2.6.1. Extraction of Soluble Protein for Enzyme Assays*

Leaf samples (1 g) from each treatment were homogenized in 2 ml of 50 mM Tris-HCl buffer (pH 7.2), containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 1% (w/v) polyvinylpolypyrrolidone (PVP) at 4°C. For ascorbate peroxidase (APX) assay, the homogenizing solution additionally contained 5 mM ascorbate. The homogenates were centrifuged at 10,000 × *g* for 15 min at 4°C and the resultant crude supernatant was collected and stored at –20°C for the determination of protein and enzyme activities. Soluble protein content in the supernatant was determined with bovine serum albumin (BSA) as the standard [38]. Antioxidant enzymes, namely, catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC. 1.15.1.1), guaiacol peroxidase (GPX, EC. 1.11.1.7), ascorbate peroxidase (APX, EC. 1.11.1.11), and glutathione reductase (GR, EC. 1.6.4.2) were determined spectrophotometrically as outlined below.

#### *2.6.2. CAT*

CAT activity [39] was determined by measuring the decrease in absorbance at 240 nm as a result of consumption of $H_2O_2$ ($\varepsilon$ = 39.4 $mM^{-1}$ $cm^{-1}$) by the enzyme, which was followed in the reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0) and 10 mM of $H_2O_2$. CAT activity was expressed in nmol of $H_2O_2$ utilized per mg of protein per min.

#### *2.6.3. SOD*

SOD activity [40] was determined by measuring inhibition of the photochemical reduction of NBT by the enzyme in the reaction mixture containing 50 mM sodium phosphate buffer (pH 7.8), 0.1 mM of EDTA, 75 µM of NBT, 13 mM methionine, 0.3% (v/v) triton X-100 and 2 µM riboflavin. The reaction was initiated at room temperature by switching on the light (Phillips 40 w × 2 fluorescent tubes) for 8 min, and stopped by switching off the light. The absorbance of formazan formed was recorded at 560 nm. One unit of SOD activity was defined as the amount of the enzyme that inhibited NBT reduction by 50% and expressed in per mg of protein per min.

#### *2.6.4. GPX*

GPX activity [41] was determined in the enzyme extract by measuring the increase in the absorbance at 470 nm due to formation of tetraguaiacol ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$) in the reaction mixture containing 50 mM of sodium phosphate buffer (pH 7.0), 10 mM $H_2O_2$, and 0.5 mM guaiacol and the activity was expressed in µmol per mg of protein per min.

#### *2.6.5. APX*

APX activity [42] was measured in the aliquot of fresh enzyme extract containing 50 µg protein that was added to a 3 ml of the reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 0.5 mM ascorbic acid and 0.2 mM $H_2O_2$. The hydrogen peroxide-dependent oxidation of ascorbate was followed by recording the decrease in absorbance spectrophotometrically at 290 nm ($\varepsilon$ = 2.8 $mM^{-1}$ $cm^{-1}$) and the enzyme activity was expressed in µmol of oxidized ascorbate per mg of protein per min.

#### *2.6.6. GR*

For the determination of GR activity [43], an aliquot of the extract containing 50 µg protein was added to a 3 ml reaction mixture containing 0.2M Tris-buffer (pH 7.8) 2mM EDTA, 0.5mM GSSG (oxidized glutathione), and 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH, HiMedia, Mumbai, India). The reaction was initiated by the addition of NADPH at 25°C. GR activity was followed by the decrease in absorbance spectrophotometrically at 340

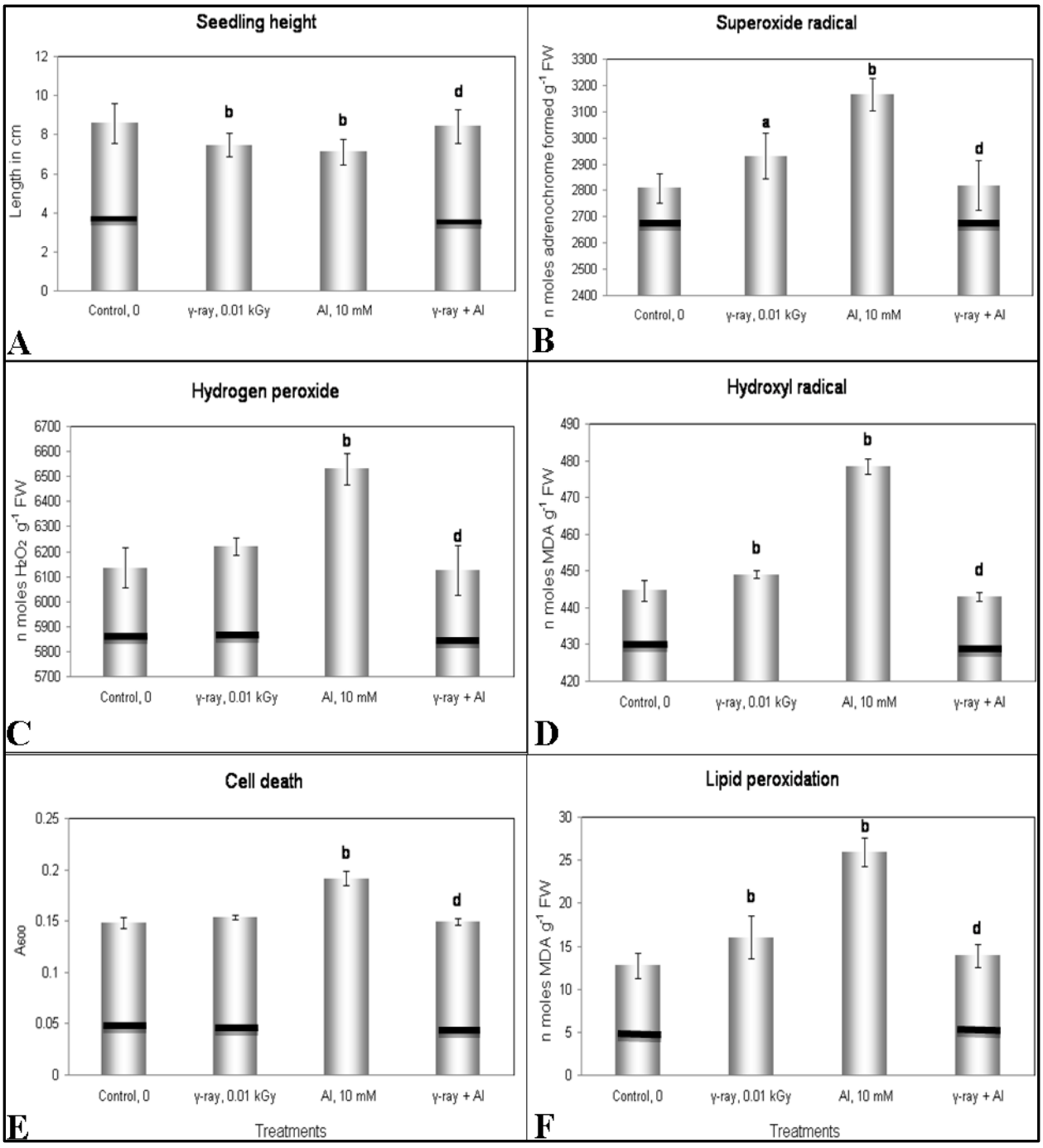


**FIGURE 1. γ-Irradiation (0.01 kGy) seed-priming modulates the $Al^{3+}$ (10 mM)-induced seedling growth inhibition (A), generation of $O_2^{\bullet -}$ (B), $H_2O_2$ (C), $OH^{\bullet}$ (D), cell death (E), and lipid peroxidation (F) as determined in *G. abyssinica* seedlings.** Significant differences were established as compared to the control at $p \leq 0.05$ (a) or 0.01 (b), and compared to the Al-treatment at $p \leq 0.01$ (d). The horizontal bars indicate no significant difference between the treatments. Vertical bars indicate standard deviation (n = 6).

nm due to NADPH oxidation (ε = 6.2 $mM^{-1}$ $cm^{-1}$) and expressed in µmoles of NADPH oxidized per mg of protein per min.

### 2.7. Chromosome Aberration and Micronucleus Assay

For the preparation of cytological slides, the root meristems from un-irradiated control and irradiated germinating seeds of plants were washed in distilled water and hydrolyzed in 1 N HCl at 60° C for 10 min, and stained with hematoxylin [44]. Slides were then scored blind under a Zeiss microscope at 400× for scored for mitotic index (MI, percent of mitosis), chromosome aberrations (CA): ana-telophases with bridge and/or fragment(s) and interphases with micronucleus (MN). At least 10,000 cells from 9 root meristems per dose were scored to determine the mitotic index (percent of mitoses) and the frequency of cells with CA (calculated per 100 ana-telophases) and MN (calculated per 1,000 interphases).

### 2.8. Statistical Analysis

All the experiments were replicated at least three times. Pooled data were subjected to analysis of variance (ANOVA), followed by the Tukey's honestly significant difference (HSD) test [45]to determine the levels of significance set at $p \leq 0.05$ or 0.01. Windows-Microsoft Excel 2003 software was employed for computation, data analysis, and graphical representation.

## 3. RESULTS

The effective priming dose of γ-irradiation of 0.01 kGy and optimum $Al^{3+}$ dose of 10 mM were selected from the preliminary studies on the establishment of the effective priming dose of γ-irradiation and optimum treatment dose of $Al^{3+}$. The results (**Figure 1**) indicated that the seedlings germinated either from the γ-irradiation primed or Al-treated seeds showed significant inhibition ($p \leq 0.01$) of seedling height in comparison with the control seedlings. This was in contrast with the seedlings grown from the seeds that were consecutively primed with γ-irradiation (0.01 kGy) and treated with $Al^{3+}$ (10 mM) which exhibited no inhibition of seedling growth (**Figure 1A**). These findings were corroborated with the extent of Al-induced oxidative stress as marked by the generation of ROS including $O_2^{\bullet-}$, $H_2O_2$, and $OH^{\bullet}$ (**Figure 1B–**

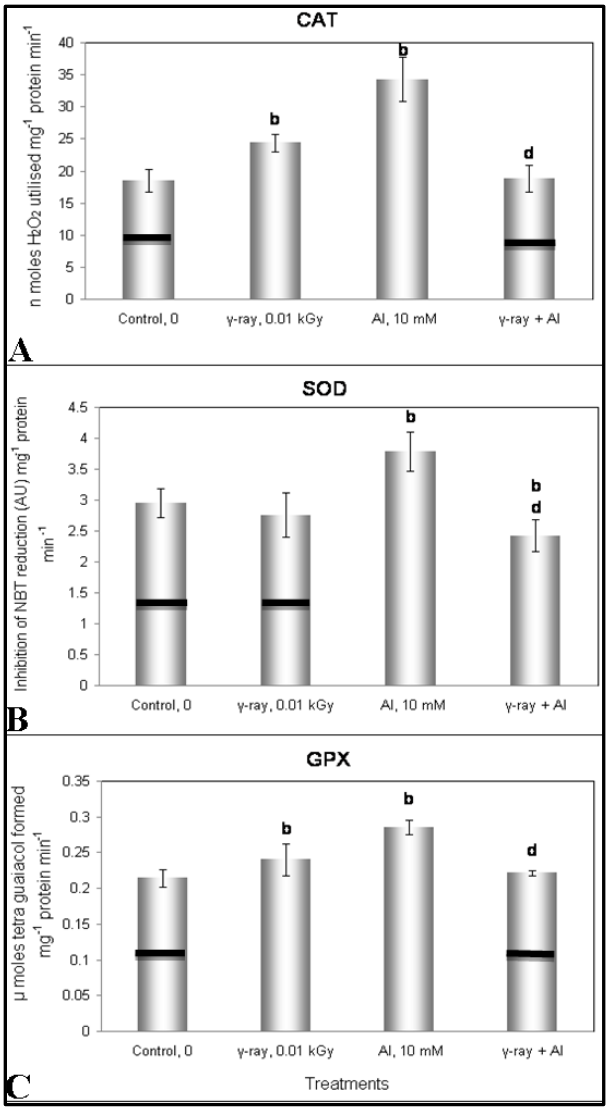


**FIGURE 2. γ-Irradiation (0.01 kGy) seed priming modulates the induction of activities of catalase (A), superoxide dismutase (B), and guaiacol peroxidase (C) induced by $Al^{3+}$ (10 mM) as determined in *G. abyssinica* seedlings.** Significant increase compared to the control at $p \leq 0.01$ (b) and significant decrease compared to the Al-treatment at $p \leq 0.01$ (d). The horizontal bars indicate no significant difference between the treatments. Vertical bars indicate standard deviation (n = 6).

1D), induction of cell death, (Figure 1E) and lipid peroxidation (Figure 1F). Seed priming with γ-irradiation invariably ameliorated the Al-induced oxidative stress in the seedlings. The seedlings grown from seeds primed with γ-irradiation revealed significant induction of CAT, GPX (Figure 2) and APX (Figure 3) activities as compared to the concurrent negative controls. Al-treatment (positive control), on the other hand, showed significant induction of all the antioxidant enzymes in the seedlings grown from the un-primed seeds. Remarkably, the seedlings grown from the seeds that were first primed with γ-irradiation at 0.01 kGy and then consecutively challenged by Al 10 mM failed to reveal any induction of the antioxidant enzymes (Figures 2 and 3).

The roots excised and fixed from the germinating seeds were analyzed for genotoxicity as marked by the mitotic index (MI), frequencies of anaphase-telophase with chromosome aberration (CA), and cells in interphase with micronucleus (MN) that were scored for each treatment (Table 1). The results on MI revealed that compared to the negative control (MI = 9.55) both γ-irradiation 0.01 kGy and $Al^{3+}$ 10 mM significantly suppressed the MI to different extent. $Al^{3+}$ 10 mM, the served as the positive control suppressed mitosis (MI = 5.09) more effectively than γ-rays 0.01 kGy (MI = 7.52). The genotoxicity analysis revealed that γ-rays 0.01 kGy induced a marginal increase in the frequencies of ana-telophases with CA or interphases with MN over the negative control, which however was statistically insignificant. On the other hand, $Al^{3+}$ at 10 mM (positive control) induced ana-telophases with CA and interphases with MN, respectively, in 3.7% and 4.37‰ frequencies that were significantly higher ($p \leq 0.01$) compared to the negative control. The roots fixed from the germinating seeds that were previously primed with γ-irradiation 0.01 kGy and challenged consecutively by $Al^{3+}$ 10 mM revealed cells with CA (2.31%) and MN (0.8‰) in frequencies that were significantly less than the corresponding values determined in the positive control. The priming of the seeds with γ-irradiation (0.01 kGy) thus conferred protection against the genotoxicity of $Al^{3+}$ (10 mM).

## 4. DISCUSSION

Seed-priming is often implicated in improving the stress-tolerance of germinating seeds, cellular mechanism of which is not well understood [16]. Furthermore, γ-irradiation at low doses can stimulate the plant tolerance to environmental stress due to salt [29], ozone [30], heavy metals [46], and heat [47]. In the current study, seed-priming by γ-irradiation at 0.01 kGy was investigated for alleviation of Al-stress in the oil seed crop, niger, *G. abyssinica* through the

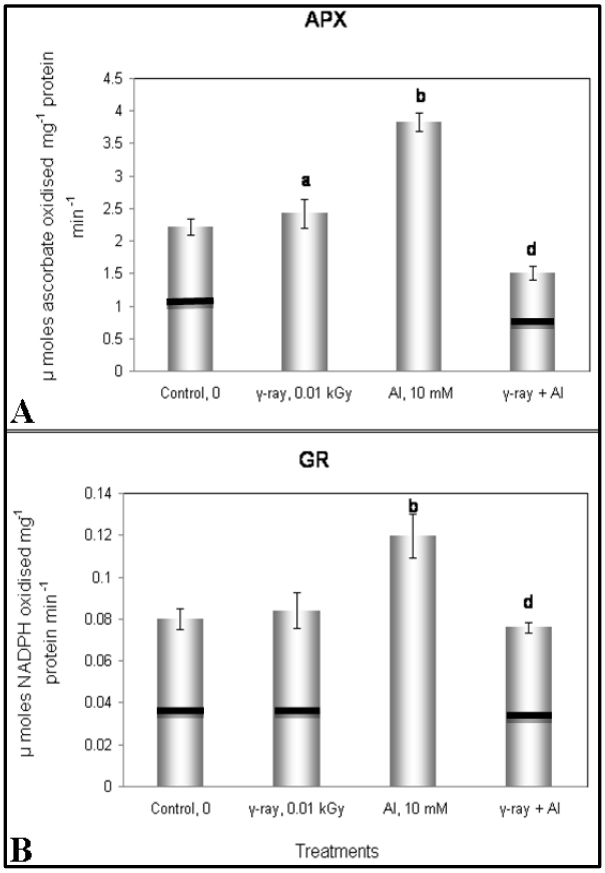


**FIGURE 3. γ-Irradiation (0.01 kGy) priming of seeds modulates the induction of activities of ascorbate peroxidase (A) and glutathione reductase (B) induced by $Al^{3+}$ (10 mM) as determined in *G. abyssinica* seedlings.** Significant increase compared to the control at $p \leq 0.05$ (a) or 0.01 (b), and significant decrease compared to the Al-treatment at $p \leq 0.01$ (d). The horizontal bars indicate no significant difference between the treatments. Vertical bars indicate standard deviation (n = 6).

**TABLE 1. Effect of seed priming with γ-irradiation (0.01 kGy) on the induction of genotoxicity by $Al^{3+}$ (10 mM) as determined by the mitotic index (MI) and the frequencies of ana-telophases with chromosome aberrations (CA) and interphases with micronucleus (MN) in the root meritems of *G. abyssinica***

| Dose/ treatment | Roots analysed | Number of cells examined | | | Mitotic index | Cell with aberrations | |
|---|---|---|---|---|---|---|---|
| | | Total cells | Ana-telophases | Interphases | | CA% | MN‰ |
| Negative control (0) | 9 | 11192 | 432 | 10124 | 9.55 ± 0.12 | 1.85 ± 0.43 | 0.58 ± 0.20 |
| γ-ray, 0.01 kGy | 9 | 11003 | 380 | 10175 | 7.52 ± 0.12[b] | 2.35 ± 0.57 | 0.88 ± 0.26 |
| Positive control $Al^{3+}$, 10 mM | 9 | 11370 | 217 | 10791 | 5.09 ± 0.16[b] | 3.75 ± 0.82 [b] | 4.37 ± 0.41[b] |
| γ-ray + $Al^{3+}$ | 9 | 11108 | 348 | 10159 | 8.54 ± 0.06 [b,d] | 2.31 ± 0.47[c] | 0.80 ± 0.19[d] |

Note: Data represent average ± standard error of mean. Significant decrease or increase at $p \leq 0.01$ (b) compared to the negative control (0), increase or decrease at $p \leq 0.05$ (c) or 0.01 (d) compared to the positive control ($Al^{3+}$, 10 mM).

determination of several biological endpoints ranging from seedling height, ROS generation ($O_2^{\bullet-}$, $H_2O_2$ and $OH^{\bullet}$), cell death, lipid peroxidation, (**Figure 1**), antioxidative enzyme activities (CAT, SOD, GPX, APX, and GR) (**Figures 2** and **3**), to genotoxicity (**Table 1**). Both γ-irradiation 0.01 kGy and $Al^{3+}$ 10 mM, the latter relatively being more effective, inhibited seedling growth marked by increased ROS, cell death, lipid peroxidation levels with concomitant increase in the activity antioxidant enzymes indicating oxidative stress [3, 48]. Dry-priming of seeds with ascorbic acid, a known scavenger of $H_2O_2$, is reported to alleviate Al-stress in *Zea mays* genotypes, which however showed a decrease in GPX activity [49]. Al-induced phytotoxicity has been attributed to the apoplastic oxidative burst taking place at the cell wall/plasma membrane interface [10]. The overproduction of $O_2^{\bullet-}$ involving the cell wall NADH-peroxidase/plasma membrane NADPH-oxidase is one of the earliest products of oxidative burst in responding to Al-stress. In turn, $O_2^{\bullet-}$ may be converted to $H_2O_2$ by SOD while $H_2O_2$ is further scavenged mainly by APX and CAT [18]. Seed-priming with $H_2O_2$ alleviated Al-induced oxidative stress in wheat seedlings that decreased the levels of ROS and lipid peroxidation through induction of the antioxidative enzyme activities to higher levels [50].

In the present study, alleviation of Al-stress by seed-priming with γ-irradiation at a low dose was evident from the fact that the seedlings grown from the γ-ray primed-Al challenged seeds retained normal growth in par with the seedlings in the negative control that showed neither any elevation of ROS levels nor induction of any antioxidant enzyme that pointed to the prevalence of normal redox state without any oxidative stress (**Figures 1–3**). Furthermore, in an earlier study, we showed that low dose γ-irradiation of seeds increased protein- and non-protein thiol contents in the seedlings of *G. abyssinica* suggesting their role in the mitigation of the oxidative stress [51]. It, therefore, seemed plausible that the mild oxidative stress generated through priming of seeds with γ-irradiation at a low dose (0.01 kGy) elicited an adaptive response that not only thwarted Al-stress but down regulated the ROS formation to such a critical low level that eliminated the need for any induction of the antioxidative enzymes (**Figures 2** and **3**). Much evidence has recently emerged that $H_2O_2$, the freely diffusible and relatively long-lived ROS, plays a dual role for defense as well as damage in plants. At low concentrations, $H_2O_2$ acts as an important signaling molecule involved in signal transduction triggering tolerance against various biotic and abiotic stresses, whereas at high concentrations it is geno-

toxic and may trigger programmed cell death [52, 53].

The genotoxicity of $Al^{3+}$ in a range of concentrations 0.01–10 mM at pH. 4.5 has been established in *Vicia faba* root meristrem assay [14]. In the current genotoxicity experiment (**Table 1**), it was established that the mitotic cell cycle in the root meristems progressed normally, ruling out any mitotic cell cycle delay as result of seed priming with γ-irradiation at 0.01 kGy ruling out any DNA damage response [54]. Whereas the genotoxicity evaluated on the basis of the frequencies of mitoses with CA or MN in the root tissue induced by $Al^{3+}$ at 10 mM (positive control) was significant, prior priming of the seeds with γ-irradiation at a low dose (0.01 kGy) conferred significant protection from any induction of genotoxic damage by $Al^{3+}$ at 10 mM. Several recent studies have demonstrated that the seed-priming not only conferred abiotic stress tolerance [18] but also ensure genomic protection from genotoxic stress possibly by triggering multiple stress-responsive pathways possibly involving gene expression and DNA repair [14, 28, 55]. Together, the results of the current study provided unequivocal evidence supporting that oxidative stress and genotoxicity induced by Al could be significantly alleviated upon priming the dry seeds of *G. abyssinica* with low dose γ-irradiation.

## 5. CONCLUSIONS

Keeping in view the fact acid soils contaminated with $Al^{3+}$ has been limiting crop yield and productivity, phytotoxicity and genotoxicity $Al^{3+}$ at 10 mM dose at pH 4.5 was assessed using the *G. abyssinica* bioassay system. The findings underscored the $Al^{3+}$-induced oxidative stress and genotoxicity in the seedlings or the germinating seeds. Importantly, prior priming of *G. abyssinica* seeds with a low dose of γ-irradiation significantly ameliorated the Al-induced phytotoxicity and genotoxicity. More noticeably, priming of seeds with low dose γ-irradiation not only conferred genomic protection but also reduced the oxidative stress when subsequently challenged with Al, which appeared to be a ROS-mediated adaptive response. The present study established that priming of *G. abyssinica* dry seeds with γ-irradiation invoked an adaptive response triggering mild oxidative stress that apparently ameliorated tolerance of the plant to Al toxicity.

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