Salicylic Acid and Malic Acid Together Alleviates More Effectively Al-induced Toxicity in Finger Millet (*Eleusine coracana* L.) Cultivars

*S. Hemalatha and Balaji Meriga*

Department of Biochemistry, Sri Venkateswara University, Tirupati, A.P., India

**Abstract:** To understand Al toxicity and tolerant mechanisms in plants, several studies have been conducted, but they are inconclusive. In the present work, the alleviating effect of salicylic acid (SA) and malic acid (MA) on Al-induced toxic effects in two finger millet (*Eleusine coracana* L.) cultivars, IE 3618 (Al-resistant) and Ratnagiri (Al-sensitive) was evaluated. Two-week old seedlings were divided into different groups and grown in growth medium (1/10th Hoagland solution) alone or in the presence of Al or SA or SA+MA in the growth medium for three days. Al treatment (100µM) caused 40% - 65% drop in plant growth, relative water content (RWC) and 2-3 folds increase Al uptake, 2-5 folds raise in malondialdehyde (MDA) levels, 0.5-2 folds increase in proline content when compared to their normal control plants. Elevated activity of superoxide dismutase (SOD) and Peroxidase (POD) were observed while catalase (CAT) activity was reduced under Al stress. Al also caused 2-5 folds decrease in the plant concentrations of Ca, K, Mg and P. External supplementation of SA and MA together has greatly restored the Al-induced alterations in the said parameters than when SA was administered alone and our study confirmed that IE 3618 is Al-resistant than Ratnagiri.

**Key words:** Proline content · Detoxification · Malondialdehyde · Antioxidant Enzymes

**INTRODUCTION**

Aluminum (Al) ranks third in abundance among the Earth’s crust elements, after oxygen and silicon. A large amount of Al is incorporated into aluminosilicate soil minerals and very small quantities appear in the soluble form, capable of influencing biological systems [1]. Al is a major limiting factor of agriculture productivity in acidic soils (pH < 5.0) which constitute about 40% of world’s arable lands. Moreover, some agricultural practices like removal of products from the farm, leaching of nitrogen below the plant root zone, inappropriate use of nitrogenous fertilizers and build-up in organic matter, are causing further acidification of agricultural soils [2]. As a result, the production of staple food crops including grains and millets are considerably limited by acidic soils. Finger millet (*Eleusine coracana* L.) is a tropical crop, belongs to the group of minor cereals. It is mainly produced and consumed in Asian and African countries. Because of the excellent storage properties of the grain, higher mineral content and the nutritive value, which is higher than that of rice and equal to that of wheat [3], finger millet is a crop that cannot be neglected. It is also a good source of micronutrients like calcium, iron, phosphorus, zinc and potassium [4].

When the soil pH falls below 5.0, Al speciates into its ionic forms, among which Al$$^{3+}$$ is the most toxic form. Apart from Al$$^{3+}$$ cation, Al has the potential to form hydroxyl-Al and polynuclear species in solution. The primary response to aluminum stress occurs in the roots [1,5]. The root system as a whole is affected, with many stubby lateral roots and no fine branching. Such roots are inefficient in absorbing nutrients and water [6]. Leaf RWC (Relative water content) could be considered as a valuable parameter to determine plants’ water status [7]. Reduced RWC could be a result of lower water availability under drought, saline conditions [8]. It may also a consequence of inefficient root system which could not retrieve the water losses because of decreasing its absorbing surface [9]. Measurement of RWC indicates the intensity of water stress experienced by a plant under Al stress. Al$$^{3+}$$ binds with oxygen donor molecules such as proteins, phospholipids, polysaccharides, nucleic acids, etc. and triggers an increased production of reactive oxygen species (ROS) which include singlet oxygen ($$^{1}\text{O}_2$$), superoxide radical ($$\text{O}_2^-$$), hydroxyl radical (OH) and...
hydrogen peroxide (H₂O₂) in the tissue. These ROS cause oxidative damage to cellular organelles and biomolecules and thus lead to several metabolic alterations [10,11]. To keep the cellular level of ROS under control and to avoid oxidative damage, plant system is endowed with antioxidant system which include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POD, EC 1.11.1.7) [12]. Malondialdehyde (MDA) is an ultimate product formed as a result of lipid peroxidation and its concentration expresses the degree of membrane peroxidation and consequent damage. Therefore, the examination of antioxidant enzyme activities and MDA content often serve as key biochemical indicator to assess the sensitivity of plants under stress conditions.

Different plant species exhibit distinct variations in regard to sensitivity and/or tolerance to Al³⁺. Mechanisms of Al tolerance have been broadly classified as those which prevent Al uptake by roots and those which detoxify the already accumulated Al in the cell. Hematoxylin staining has been used as a precocious, non-destructive way to study Al sensitivity in plant species [13,14]. In maize, this staining has also been used as a selection phenotypic index for Al resistance [15,16].

Proline as an osmoregulatory solute is thought to play a prime role in plants growing under stress conditions like water deficit and soil salinity. Indeed, the accumulation of this amino acid may be part of a general adaptation to several environmental stresses including exposure to Al. Since plants subjected to Al stress undergo similar physiological changes as that of plants exposed to drought stress, the role of proline is implicated as an adaptive measure to prevail upon such stress conditions. Proline stabilizes cellular structures as well as scavenges free radicals. Al interferes with the uptake, transport and use of essential elements such as Ca, Mg, P, K and Fe and according to Foy [17], the ability to maintain higher root and shoot concentrations of macro and micro nutrients cations in the presence of Al has usually been associated with Al resistant cultivars. However, studies devoted to the influence of Al upon mineral nutrition in plants frequently gave contradictory results. In our study the concentrations of elements like Ca, Mg, K and P were analyzed under Al stress, in the absence of SA and MA, to validate its effect on other elements.

Organic acids (OA), such as citrate, malate, oxalate play an important role in plant resistance to Al stress. Exudation of different organic anions correlated with Al resistance of different plant species [18]. The existence internal detoxification of Al by organic acids is more debated in different plant species. [19] Showed that the response of Sorghum roots to Al was an increase in taconitic and malic acids (MA). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature and it could be included in the category of phytohormones, which participates in the regulation of physiological processes in plants [20]. In this study we aimed to evaluate the alleviating efficiency of SA and MA on two cultivars (IE 3618 and Ratnagiri) of finger millet differing in their sensitivity to Al stress.

**MATERIALS AND METHODS**

**Plants and Treatments:** Finger millet seeds were obtained from ICRISAT, Hyderabad and Agricultural research institute (ARI) Tirupati. After preliminary screening, two cultivars, viz, IE 3618 and Ratnagiri were identified as relatively Al-resistant and Al-sensitive cultivars. The seeds of these two cultivars were surface sterilized and germinated in petridishes. To study growth parameters and for other biochemical studies, 2 week-old seedlings were divided into different groups and grown in 1/10 th Hoagland solution. Group-1: Normal control (1/10 th Hoagland solution), group-2 (1/10 th Hoagland solution containing Al-100µM), group-3: (1/10 th Hoagland solution containing Al-100µM+SA-100µM) and group-4: (1/10 th Hoagland solution containing Al-100µM+SA-100µM+MA-100µM) for 72 h, at 25°C±2°C under 12 hr light-dark cycles and harvested for further analysis. The treatment solutions were renewed every day.

**Plant Growth and Relative Water Content Determination:** After 72 h treatment, the root and shoot lengths of normal control and treated seedlings were measured by using scale, then RWC was determined according to the procedure of Smart and Bingham using the following formula [21].

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RWC= (\text{fresh weight-dry weight/fresh weight}) \times 100.
\]

**Measurement of Al Uptake:** Hematoxylin staining procedure was used to determine Al uptake [22]. Roots of seedlings were initially washed in distilled water for 20 min followed by staining with 0.2% aqueous hematoxylin solution containing 0.02% KI for 20 min at room temperature and then washed with distilled water for 20 min. Root tips, 10-15 number, were collected and soaked in 250µL of 1 M HCl for 1 h.
The optical density (OD) of released stain was measured at 490 nm using spectrophotometer (Perkin Elmer, USA) and Al uptake was calculated.

**Determination of Lipid Peroxidation and Proline Content:**
Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) formed by the thiobarbituric acid reaction as described by Heath and Packer [23]. Leaves were ground with a pestle and mortar in 1% TCA (10 mL/g fresh weight) and centrifuged at 10,000 rpm for 5 min. To 1.0 mL of supernatant in a separate test tube, 4.0 mL of 0.5% TBA was added. The mixture was heated at 95°C for 30 min, then cooled in ice cold water and later centrifuged at 5000 rpm for 5 min. Absorbance was measured at 532 nm and the blank contained 1% TBA in 20% TCA. MDA content was calculated using an extinction coefficient of 155 mm$^{-1}$ cm$^{-1}$.

Proline content was measured as per the procedure described by Bates, Waldren and Teari [24]. Fresh leaves of finger millet treated seedlings were homogenized with sulphasalicylic acid and centrifuged at 5000 rpm. The supernatant was collected and the absorbance was measured at 520 nm. Proline content was expressed as µg g$^{-1}$ fresh weight.

**Assay of Antioxidant Enzymes:** Fresh leaves (0.5 g) were homogenized with a mortar and pestle under chilled conditions with phosphate buffer (0.1 M, pH 7.5) and ethylene diamine tetra acetic acid (EDTA, 0.5 mM). The homogenate was filtered and centrifuged at 12, 000 g for 10 min at 4°C. The supernatant was used for assay of antioxidant enzymes.

Superoxide dismutase (SOD) activity was determined by using [25] by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT). The assay mixture contained 1 mL of enzyme extract, 0.1 mL of enzyme extract, 0.1 mM phosphate buffer (pH 7.5), 3 mM NBT and 60 mM riboflavin. The tubes were thoroughly shaken and placed under 15 W fluorescent lamp for 10 min, then the lights were switched off and the tubes were covered with a black cloth. For the purpose of blank, the non-illuminated reaction mixture was used. Absorbance of reaction mixture was read at 560 nm and one unit of SOD activity (EU) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo reduction rate. The results were expressed as units mg$^{-1}$ protein.

Peroxide assay was done following the procedure described by Nakano and Asada [26]; the assay mixture contained 1.0 mL of potassium phosphate (pH 7.0) buffer supplemented with 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H$_2$O$_2$ and 0.1 mL of enzyme extract. POD activity was measured in terms of decrease in absorbance at 290 nm of ascorbate and expressed as units mg$^{-1}$ protein. For the calculation of POD activity, the extinction coefficient of 2.8 mM$^{-1}$ cm$^{-1}$ was used. One unit of enzyme activity was considered as the amount required for decomposing 1 µM of substrate per min at 25°C.

Catalase (CAT) activity was measured by using [10] method. The assay mixture contained 0.1 mL of enzyme extract, 0.1 mM phosphate (pH 7.5), 0.1 M EDTA and 0.3% H$_2$O$_2$ and the absorbance was measured at 240 nm. CAT activity expressed as units mg$^{-1}$ protein. For the calculation of CAT activity, the extinction coefficient of 0.036 mM$^{-1}$ cm$^{-1}$ was used.

**Analysis of Elements:** Ca, K, Mg and P contents were measured using an inductively coupled argon plasma optical emission spectrophotometer (Jobin-Yvon JY 48). For this analysis the fresh leaves of treated plants were digested in nitric acid/perchloric acid at a ratio of 5:1. The final solution was diluted to 10-15 ml with 0.1 N HNO$_3$, which was subjected to ICPOES and the final results obtained were expressed in µg/mg of fresh weight.

**Statistical Analysis:** Experimental results are expressed as means ± SE. All measurements were replicated six times. The data were analyzed by analysis of variance ($P < 0.05$) and the means were separated by Duncan’s multiple range test.

**RESULTS**

**Plant Growth:** Inhibition of Root length and weight followed by shoot length and weight was the earliest symptom of Al toxicity in Finger millet. In our study when compared to normal control plants, the root and shoot lengths were significantly decreased in plants treated with Al-100µM concentration. There was 20% inhibition in plant growth of IE 3618 which is a resistant cultivar while 40% inhibition in plant growth was noticed in Ratnagiri, a sensitive cultivar. Addition of SA to Al-treated seedlings slightly improved plant growth. Interestingly addition of SA and MA together substantially improved plant growth, with more profound recovery being observed in IE 3618 than Ratnagiri, as depicted in fig-1A & 1B.
Fig. 1A: Effect of Al, SA and MA on root and shoot growth of IE 3618 seedlings. The values are mean ±SE (n=5). * Indicates significant difference ($p < 0.05$) when compared with Al100µM treated seedlings.

Fig. 1B: Effect of Al, SA and MA on root and shoot growth of Ratnagiri seedlings. The values are mean ±SE (n=5).

**Relative Water Content (RWC):** Figure 2 explains the relative water content of finger millet seedlings. When compared to normal control plants, RWC of Al-treated plants was substantially low. The RWC of Ratnagiri and IE 3618 was 55% and 70% in Al-treated seedlings. However, exogenous supplementation of SA significantly improved RWC of these two cultivars to 65% and 80% respectively. Moreover, supplementation of SA and MA together greatly improved RWC (80% and 90% respectively in IE 3618 and Ratnagiri). Our observations indicate that external supplementation of SA and MA could substantially improve RWC, with IE 3618 showing better recovery than Ratnagiri under Al stress.

**Al Uptake by Plants:** Al-induced inhibition of root length indicates a marked increase in the uptake of Al by the roots grown in Al-containing solution. Spectrophotometric quantification of hematoxylin stain in root tips of two Al-treated finger millet varieties revealed 1 - 2.5 fold increase of Al uptake when compared to normal controls. The supplementation of SA and MA in the treatment medium lowered Al uptake by the roots, stain intensity and area declined considerably (0.6 - 1.1 fold) in root tips of Al + SA + MA-treated seedlings compared to Al alone treated seedlings as shown in Fig.3 and this improvement is certainly better than when SA was administered alone.
Fig. 2: Effect of Al, SA and MA on RWC of IE 3618 and Ratnagiri seedlings. The values are mean ±SE (n=5).

Fig. 3: Spectrophotometric quantification of Hematoxylin stain to find Al uptake content in finger millet cultivars. The values are mean ±SE (n=5).

**Lipid Peroxidation and Proline Content:** In the present study, greater lipid peroxidation was observed in finger millet seedlings under Al stress as evident from 0.7 and 2.1 fold higher MDA content in IE 3618 and Ratnagiri respectively, than their respective controls. Fig. 4 showed better recovery for IE 3618 from toxic effects of Al than Ratnagiri with SA and MA supplementations. Higher levels of MDA under Al stress indicates increased generation of reactive oxygen species and consequent membrane damage to membrane integrity and membrane dynamics. These results suggest that, when compared to IE 3618, seedlings of Ratnagiri have undergone higher lipid peroxidation because of their susceptible nature.

Proline is considered as a compatible osmolyte produced under various stress conditions and has been suggested to function as a scavenger of hydroxyl radicals, controlling redox homeostasis. In the present study proline content increased 0.5-fold in IE 3618 and 2.0 fold in Ratnagiri (Fig 5), in the presence of Al when compared to normal control plants. Supplementation of SA and MA has dose-dependently and significantly decreased proline synthesis in both the cultivars. Our results of decreased proline levels demonstrate that supplementation of SA and MA has greatly alleviated the toxic effects of Al.
Activities of Key Antioxidant Enzymes: In our study the activity of important antioxidant enzymes was measured in both IE 3618 and Ratnagiri cultivars in the presence and absence of Al, SA and MA, to evaluate their role in scavenging ROS. Without Al treatment, both IE 3618 and Ratnagiri leaves exhibited only basal SOD activity, with no significant difference between them. However, after 100 µM Al treatment, the SOD activity of IE 3618 and Ratnagiri substantially increased 2.0 and 4.0 fold respectively (Fig. 6 A) over their controls. However, its activity gradually decreased in these cultivars when supplemented with SA alone and SA + MA. This results shows that IE 3618 was relatively resistant to Al stress than Ratnagiri, which was evident from lesser SOD activity in the presence of MA and SA, indicating lower ROS accumulation to scavenge.

Catalase (H₂O₂ oxidoreductase) is a heme-containing enzyme that catalyzes the dismutation of H₂O₂ into H₂O and O₂. In the present study, CAT activity decreased 0.8 fold in IE 3618 and 2.0 fold in Ratnagiri under Al stress when compared to their normal control plants. With supplementation of SA to growth medium, a moderate raise in CAT activity was noticed in the finger millet cultivars. Moreover, by adding SA and MA to the growth solution 0.5-1.0 fold raise was observed (Fig. 6 B) in these cultivars. This suggested that exogenous supplementation of SA+ MA tried to enhance CAT activity in Al-exposed seedlings there by facilitating dismutation of H₂O₂ in to non toxic forms so as to minimize the ROS induced oxidative stress.

Peroxidase (POD) is an important ROS scavenging enzyme that uses ascorbate as hydrogen donor to breakdown H₂O₂ to form H₂O. In the present study, no significant change in the activity of peroxidase was observed between finger millet cultivars in the absence of Al. However, at 100 µM Al concentration, POD activity increased 2.0 and 4.0 fold in IE 3618 and Ratnagiri respectively. Upon supplementation of SA and MA to growth medium, POD activity was restored to near normally in IE 3618 but Ratnagiri continued to exhibit 0.5 fold higher activity (Fig. 6 C). The elevated levels of POD activity confirm its active role in efficient conversion of H₂O₂ in to H₂O in finger millet cultivars, thus reducing oxidative stress inflicted by Al stress.
Fig. 6C: Effect of Al, SA and MA on POD activity in finger millet seedlings. The values are mean ±SE (n=5).

**Elemental Analysis:** The concentrations of Ca, Mg, P and K were measured in all the experimental cultivars of finger millet. When compared to control plants, 10-15% reduction in the concentration of these elements was observed in IE 3618 plants while a reduction of 33-45% was noted in Ratnagiri cultivars (Table 1) under Al stress. However, supplementation of SA and MA increased the concentrations of these elements up to 20-40% in both resistant and sensitive cultivars when compared to Al treated plants.

**DISCUSSION**

Aluminum toxicity is one of the dominant environmental stress factors limiting plant growth in acidic soils. Under acidic soil conditions the phytotoxic forms of Al are released into the soil solution at levels that can inhibit root growth and plant development [27]. In the present study, the toxic effects of Al as well as their mitigation by SA and MA were examined in two finger millet cultivars having different levels of sensitivity to Al stress. After preliminary experiments, two cultivars, viz., IE 3618 and Ratnagiri were selected as relative Al-resistant and Al-sensitive plants respectively. The two finger millet cultivars were subjected to 100 µM Al stress in the presence and absence of SA and MA to evaluate their physiological and biochemical responses.

Plant growth is the combination of cell division and elongation. In the present study the root, shoot lengths and RWC of finger millet cultivars were decreased, with marked inhibition being observed with Ratnagiri than IE 3618 under Al stress (fig. 1A, 1B and 2). This could be due to increased accumulation of Al in Ratnagiri seedlings which may interfere in the normal metabolic processes in addition to enhanced ROS production [28,29]. In fact, more Al accumulation was noted in our study also in Ratnagiri cultivars. It is also documented that Al accumulation affects nutrients as well as water uptake [30]. A similar inhibitory effect of Al on root elongation was reported in other plant species including maize [31], wheat [32] and rice cultivars [33]. Long term exposure of plants to Al stress leads to decreased water uptake and decreased retention owing to interaction of Al with root cells nuclei, root cell division and cytoskeleton formation [34,35]. Lesser inhibition of plant growth and lesser accumulation of Al in IE 3618 under Al stress is possibly because of release of Al chelating molecules in to the apoplastic region of roots which might prevent Al entry in to the plant system and thus minimizes eventual damage. However, the plant growth and RWC of both the cultivars was much improved when growth medium was supplemented with SA and MA. This could be mediated through exclusion or detoxification mechanism. Supplementation of SA and MA may lead to increase in the concentration of solutes in the cell causing improved water intake, high RWC and maintenance of osmotic balance. SA supplementation might enhance auxin mediated root elongation. Similar results of reduced uptake of Al and Cd by salicylic acid was reported in rice and maize [36].

Lipid peroxidation (LPO) is an important and sensitive marker to ROS induced stress and membrane damage. Measurement of MDA levels reflects the intensity of membrane damage. It has been well quoted that Al exposure is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals. In the present study, greater lipid peroxidation was observed in Ratnagiri seedlings than IE 3618 under Al stress. Nevertheless, SA and MA supplementations resulted in better recovery of the seedlings (Fig. 4). Higher MDA content in Al-treated seedlings shows the increased production of reactive oxygen species and consequent membrane damage, loss of membrane integrity and membrane dynamics. Our observations suggest that, when compared to IE 3618, seedlings of Ratnagiri have undergone higher lipid peroxidation because of their susceptible nature. Earlier studies reported that Al caused enhanced peroxidation of lipids in cereals and legumes [37] and caused inhibition of root elongation [38,39]. Phosphatidylserine is the most susceptible substrate for Al to facilitate lipid peroxidation [40].

Proline is considered as a compatible osmolyte produced under various stress conditions and has been suggested to function as a scavenger of hydroxyl radicals
also, controlling redox homeostasis. It is reported to protect cellular and subcellular structures including enzymes [41]. As an important osmoprotectant, it aids in maintaining adequate turgor pressure for cell expansion and cell division under stress conditions [42]. In the present study, higher proline content was noticed in IE 3618 than Ratnagiri as shown in figure 5. Supplementation of SA and MA has significantly decreased proline synthesis in finger millet cultivars. Accumulation of proline has been reported from increased proline biosynthesis, lower proline utilization, decreased degradation of proline and proteins [43]. Soil acidity is reported to cause accumulation of proline in pea, soybean, wheat, barley, maize, sorghum and Polygonum [44] [45]. Guo et al. [45] reported that Al and Cd toxicity caused increase in proline levels in barley seedlings. In another study, Al decreased proline accumulation in leaves of *Sorghum bicolor* plants ([46]. It is difficult to pinpoint the exact role of proline under the aluminum toxicity. Proline was also mentioned to activate antioxidant defense system which may be also responsible for better growth of IE 3618 cultivars. Hence, higher proline accumulation is quite often implicated in enhanced Al tolerance in plants.

Production of ROS is enhanced under different environmental stress conditions. The most effective antioxidative enzyme system involved in removing ROS induced cellular damage includes SOD, POD and CAT. Super oxide dismutase (SOD) catalyzes the conversion of \( \cdot O \) to \( \cdot O_2 \), whereas CAT scavenges \( \cdot H_2O_2 \) and peroxidase uses \( \cdot H_2O \) for the oxidation of various inorganic and organic substrates. In the present study, SOD and POD activities increased while CAT activity decreased substantially under Al stress in both the finger millet cultivars. An increase in SOD activity is often correlated with increased rate of removal of \( \cdot O \) from cells in order to keep \( \cdot O \) level under control. Increase in \( \cdot O \) level under Al stress could necessitate higher SOD activity in Al-treated plants. PODs are regarded as stress enzymes and the activity of peroxidases increases under abiotic stresses including Al toxicity [47]. In the present study, when compared to IE 3618, higher SOD and POD activity noticed in Ratnagiri indicates their need to scavenge higher levels of oxygen and peroxide radicals formed in the later cultivar (fig. 6 A and 6 C). A similar trend in CAT activity was earlier reported in garlic roots when exposed to Pb, indicating the inactivation of enzyme due to accumulation of \( \cdot H_2O_2 \) induced by Pb [48]. When SA and MA was supplemented to Al containing medium, it caused fall in SOD activity but stimulated Al-led rise in CAT activity revealing reduced levels of oxygen free radicals. This suggests that SA and MA have roles in alleviation of Al induced damage by adequately maintaining the activity levels of the antioxidative enzymes. In other words, they suppress the production of \( \cdot O \) by a protective mechanism which in turn results in relatively low level of SOD and POD activities [49]. It is proposed that, after binding with SA, CAT becomes inactive leading to \( \cdot H_2O_2 \) accumulation in plant cells [50]. However, in our studies, in Al+SA+MA treated plants, CAT activity showed a gradual increase with supplementation of SA and MA in the growth medium. IE 3618 showed maximum recovery in its CAT activity while Ratnagiri could not. Restoration of POD activity to the normal level in finger millet plants appears to be a characteristic feature of SA and MA in Al treated seedlings. In other studies, treatment with SA alleviated mercury toxicity in alfalfa plants by preventing oxidative stress in roots and increasing the activities of NADH-oxidase, APX and POD [51].

Aluminum stress leads to nutritional deficiencies in plants [52] [53]. In our study we analyzed the concentrations of Ca, K, P and Mg in both resistant and sensitive cultivars with ICPOES. We noticed significantly reduced levels of Ca, K, P and Mg in both the cultivars under Al stress. This shows that Al toxicity considerably disturbs the homeostasis of other important elements. However, exogenous supplementation of SA and MA significantly restored the levels of the said nutrients (Table 1). This suggests that SA and MA play a vital role

<table>
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<th>Element</th>
<th>Control (mg/Kg leaf)</th>
<th>Al-100µM (mg/Kg leaf)</th>
<th>Al-100µM+SA-100µM (mg/Kg leaf)</th>
<th>Al-100µM+SA-100µM+MA-100 µM (mg/Kg leaf)</th>
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The values are mean ±SE (n=5). Values showing different superscripts represent significant difference (\( p<0.05 \))

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in protecting from Al toxicity and in maintaining mineral and water homeostasis in the seedlings. In other study, exogenous application of succinic acid mitigated Al-induced inhibition of mineral nutrient absorption (K, Ca, Mg, Mn and Zn) [54].

CONCLUSION

In the present work, we report that, Al stress substantially inhibited plant growth, relative water content and the tissue levels of Ca, K, P and Mg. Al enhanced ROS generation, LPO, proline content and the activity of SOD and POD. The toxic effect of Al was more pronounced on Ratnagiri than IE 3618 which demonstrates that the latter cultivar was relatively resistant. External supplementation of SA and MA together has substantially restored the Al-induced alterations in the said parameters than when SA was administered alone, revealing their synergistic effect.

REFERENCES


