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5-AMINOLEVULINIC ACID AMELIORATES SALINITY-MEDIATED GROWTH, PYSIOLOGICAL AND BIOCHEMICAL CHANGES IN MUSTARD (*Brassica juncea* L.)

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Abstract

5-aminolevulinic acid (ALA) is an important plant growth regulator which is derived from 5-carbon aliphatic amino acid. Here, ALA was examined in 12 dSm^{-1} salinity in growth and biochemical changes in mustard (*Brassica juncea* L. cv. BARI Sarisha-16) seedlings. Three NaCl mediated salinity levels (0, 8 and 12 dS m^{-1}) were used with two ALA concentrations (30 and 60 mgL^{-1}). Saline stress decreased plant height, root length, leaf area, dry mass accumulation, chlorophyll content, photosynthetic parameters, and K^+, while proline (Pro), Na^+, Na^+/K^+ ratio, antioxidant enzymatic activities, hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and melondialdehyde (MDA) content increased. Saline stressed mustard seedlings treated with 30 mgL^{-1} and 60 mgL^{-1} foliar application of ALA ameliorated the saline mediated inhibition in seedling growth i.e. increased plant height, root length, leaf area, and dry matter. ALA had also increase chlorophyll (Chl) content, net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*) and transpiration rate (*Tr*), proline content as well as the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). In contrary, ALA decreased saline induced H_2O_2, $O_2^{\bullet-}$, and MDA while Na^+ and Na^+/K^+ ratio increased. On the basis of the results, it was observed that ALA is a promising plant growth regulator which can improve plant survival under salinity.

Keywords: 5-Aminolevulinic acid; Salinity; Antioxidant enzymes; Growth and physiological parameters; Ionic balance; *Brassica juncea* L.

Introduction

Salinity is one of the major abiotic stress which affects the growth and productivity of many crops especially Oilseed crops *(Brassica juncea* L.). Changes in different morphological, physiological, biochemical and metabolic processes, however, are the most common instances under excessive salt stress, depend on the period and extent of stress and consequently impede agricultural

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production. Numerous changes have occurred in plants subjected to salinity stress, including membrane destruction, nutrient disparity, capability impairment to detoxify reactive oxygen species (ROS), decreased photosynthetic function, variations in enzymatic activity of antioxidants, and decreased aperture of stomata (Apel and Hirt, 2004; Rohman *et al*., 2016). Finally, salinity in soil ultimately suppresses the growth of most plants. However, different plants adopt different strategies to counteract these salinity-induced adverse effects.

Recent research has found that plant growth regulators (PGRs) are known to play a vital role in mitigating the adverse effects of saline stress on plants (Akram *et al.,* 2012). 5-aminolevulinic acid (ALA), is a key precursor in the biosynthesis of porphyrin-derivatives biosynthesis and is widely found in plants, especially in non-protein amino acids in living cells. ALA exhibits PGR properties, promoting plant growth, development and numerous physiological responses under normal environments and stressful conditions when applied at low concentrations (Yang *et al.,* 2014). It is also involved in important plant physiological functions, including respiration, photosynthesis, and other metabolic activities (Zhang *et al.,* 2015). Foliar application of ALA at low concentrations increases leaf chlorophyll content, promoting photosynthetic capacity of plants (Youssef *et al.,* 2010). ALA enhanced accumulation of K^+ and maintenance of high K^+/Na^+ ratio in the root and leaves of sunflower (Akram *et al.,* 2011), and to improve saline tolerance in cotton seedlings, and to reduce accumulation of Na^+, and ionic effect in plants under saline conditions (Watanabe *et al.,* 2000). Improved salinity tolerance with ALA application has also been reported mostly in many plant species (Yang *et al.,* 2014 and Zhang *et al.,* 2015). Nonetheless, underlying mechanisms remain to be established as to how ALA stimulates antioxidant systems to modulate plant stress resistance under the combination of salt stresses.

Oilseed *B. juncea* is one of the most important sources of edible oil in the world as well as in Bangladesh. Out of 2.83 million hectares of the coastal areas of Bangladesh about 0.88 million hectares are affected by salinity (Rohman *et al.,* 2019). There is a vast coastal area in the southern part of Bangladesh exhibit soil salinity of various magnitudes due to rush of saline water or flash flood from the Bay of Bengal. So, introduction of saline tolerant mustard varieties and foliar application of ALA may play an important role in enhancing crop production in these areas. The present research was under taken to investigate the alleviating role of ALA ameliorating of salinity stress in mustard cultivation.

Materials and methods

Plant materials and stress treatments

An experiment was carried out in a net house of Oilseed Research Centre at Bangladesh Agricultural Research Institute (BARI), Gazipur during 2018-19, and

biochemical and antioxidant data were analyzed in Molecular Breeding Lab, Plant Breeding Division, BARI. Healthy seeds of a *Brassica juncea* variety, BARI Sarisha-16, were obtained from the Oilseed Research Centre, BARI. Seeds were surface sterilized with 1% sodium hypochlorite solution for 10 minutes and were vigorously rinsed with distilled water. The plastic pots were filled with sandy loam soil (pH 6.8) and seeds were sown. Full strength Hoagland's nutrient solution was applied on alternate days to each pot. The pot experiment was conducted following completely randomized design with three replications. After 8 days of germination, seedlings were thinned to maintain nine plants in each experimental unit. After two week acclimatization period, solutions were adjusted to the desired salinities (0, 8, and 12 dSm^{-1}) in Hoagland nutrition media, and plants were simultaneously treated with an aqueous solution of 5-Aminolevulinic acid (ALA, sigma, USA) at a concentration of 30 mg L^{-1} and 60 mg L^{-1} by foliar spray. In control treatment, only Hoagland's nutrient solution was added (without salinity solution). Following were the treatment combinations viz (A_0S_0, control; A_0S_1, 8 dSm^{-1} salinity; A_0S_2, 12 dSm^{-1} salinity; A_1S_0, 30 mg L^{-1} ALA; A_1S_1, 30 mg L^{-1} ALA + 8 dSm^{-1} salinity; A_1S_2, 30 mg L^{-1} ALA + 12 dSm^{-1} salinity, A_2S_1, 60 mg L^{-1} ALA + 8 dSm^{-1} salinity; A_2S_2, 60 mg L^{-1} ALA + 12 dSm^{-1} salinity). Salinity levels were measured during each saline application time by direct soil EC meter (Spectrum-2265FSTP, USA). A subsequent application was made 3 days after first spray. Control treatments were also maintained under same condition. Twenty eight days after ALA treatments, six plants per replication were harvested and separated into shoots and roots. The samples were then oven-dried at 80°C for four days to record dry masses. Photosynthesis data were taken at net house. Other plants of each treatment were separately harvested for recording data in leaves for the following variables:

Leaf area

Leaf area was measured by using a leaf area meter (Li-3100, USA). Leaves of five plants for each treatment were directly used into the machine, and data were obtained as cm^2.

Chlorophyll content

The chlorophyll (Chl) content was measured following the method of Arnon (1940) after extraction of plant leaves by 80% v/v acetone. Absorbance was measured by UV-vis spectrophotometer (Shimadzu UV-1800, Japan).

Photosynthetic parameters

Gas exchange parameters were determined following Tian-gen *et al.* (2017) using a LiCOR 6400 open system portable infrared gas analyzer (IRGA) (Lincoln, USA).

Inorganic sodium (Na^+) **and potassium** (K^+) **ions**

To assess the Na^+ and K^+ ions contents, the sap was extracted from freshly harvested shoots, using a tissue sap extractor (LAQUA twin, Horiba, Japan), following the methods stated in Rohman *et al.* (2016).

Proline (Pro) content and melondialdehyde (MDA) content

Pro content in mustard leaves was measured according to Bates *et al.* (1973) based on proline's reaction with ninhydrin. MDA content was determined following to the method described by Rohman *et al.* (2016).

Superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) content

Superoxide and H_2O_2 content were measured according to the method described by Rohman *et al*. (2016). Superoxide and H_2O_2 were calculated from a standard curve of $NaNO_2$.

Protein estimation

The protein concentrations in the leaves extract were determined according to the method of Bradford (1976) using serum from bovine albumin (BSA) as a protein standard.

Extraction of soluble protein

Using a pre-cooled mortar and pestle, 0.5 g of fresh leaf tissue was homogenized in 0.009 g ascorbic acid, 10 ml of 500 mM ice-cold potassium-phosphate buffer (K-P buffer, pH 7.0), 5 mL KCl in 1 M solution, 5 mM 25 µl β-marcapto ethanol, 10% (v/v) glycerol, and volume up to 50 ml. The homogenates were centrifuged at 11500×*g* for 15 min at 4°C, and the supernatants were used for determination of enzyme activity. All procedures were performed at a temperature 0-4°C.

Assay of enzymatic activities

The inclusive procedures mentioned by Rahman *et al.* (2016) have been used for preparing the enzyme extracts and determining the activities of superoxide dismutase (SOD; EC: 1.15.1.1), catalase (CAT; EC: 1.11.1.6), ascorbate peroxidase (APX; EC: 1.11.1.11) and peroxidase (POD; EC: 1.11.1.7).

Statistical analysis

All data obtained was analyzed by R (Version 3.5) program following complete randomized design. Values of mean ± SE were calculated from three replications, and $p \leq 0.01$ and $p \leq 0.05$ were considered as level of significance.

Results and Discussion

Effect of ALA on biomass accumulation of *Brassica juncea* seedling under saline stress

Salinity treatments affected the growth of mustard seed variety which was more affected by higher salinity. The phenotype of the seedlings also showed that ALA application bettered the growth of the seedlings (Photoplate 1).

Photoplate 1. Effect of ALA on growth of *B. juncea* seedling under saline stress.

The effects of different treatments of ALA and salinity on plant growth parameters such shoot length, root length, fresh weight, dry weight and leaf area per plant are given in Table 1. The detrimental effect of salinity in growth parameters increased with salinity level as compared to control (A_0S_0). Salinity of 12 dSm^{-1} (A_0S_2) significantly decreased shoot length (56.15%), root length (57.99%), fresh weight (58.53%), dry weight (68.89%) and leaf area (63.57%) of mustard seedling compared to their respective control. Foliar application of ALA markedly increased the plant growth parameters under saline stress condition. As compare to A_0S_2, A_1S_2 was better to increases the shoot length (65.76%), root length (116.82%), fresh weight (106.5%), dry weight (172.78%) and leaf area (136.67%) (Table 1). However, ALA treatment with 60 mgL^{-1} ALA and 12 dSm^{-1} salinity (A_2S_2) decreased biomass accumulation compared to the treatment at 30 mgL^{-1} ALA and 12 dSm^{-1} salinity, respectively.

According to the results, we found that salinity causes significant effects on growth of *B. juncea*. Salinity stress changes the thermodynamic equilibrium of water and ions. As a consequence, the water potential of the rhizosphere decreases and difficulties in root water uptake occur. Thus, the root absorption capability is reduced, and the supply of water from roots to shoots is decreased. Plant roots under salinity stress are often the first to experience stress and produce corresponding physiological responses. Therefore, root and shoot growth were inhibited. As shown in Table 1, ALA treatment can alleviate salinity stress during the growth of *B. juncea* seedlings, improve dry matter accumulation, promote plant growth, and relieve salinity stress damages. This increase in growth parameters are caused by ALA treatment, which improves day time photosynthesis and reduces night time respiration (Zhang *et al.*, 2015).

At the same time, *B. juncea* treated with a foliar application of ALA experienced significantly improved growth when under saline stress. The effect might be linked to the fact that ALA has a promotive role in regulating a number of metabolic processes, thereby improving the growth and yield of most plants under abiotic stresses (Akram *et al.*, 2012). Our results were also corroborated with the previous growth by ameliorating the effect of a salinity stress.

Table 1. Effect of 5-aminolevulinic acid (ALA) on saline-induced changes in shoot length, root length, fresh weight, dry weight and leaf area in *B. juncea* seedling.

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Leaf area (cm^{2}plant^{-1})
A_0S_0	48.89^{a}	18.26^{a}	48.57^{a}	10.16^{a}	265.35^{a}
A_0S_1	28.21^{cd}	11.56^{b-d}	33.31^{a-c}	6.53^{a-c}	154.87^{bc}
A_0S_2	21.44^{d}	7.67^{d}	20.14^{c}	3.16^{c}	96.39^{c}
A_1S_0	45.63^{ab}	17.87^{ab}	46.64^{a}	9.05^{ab}	267.36^{a}
A_1S_1	29.72^{b-d}	11.36^{c-d}	30.13^{bc}	6.92^{a-c}	194.75^{a-c}
A_1S_2	35.54^{a-c}	16.63^{a-c}	41.59^{ab}	8.62^{ab}	228.13^{ab}
A_2S_1	30.49^{a-d}	15.34^{a-d}	34.45^{a-c}	7.68^{ab}	154.36^{bc}
A_2S_2	26.41^{cd}	12.48^{a-d}	30.41^{bc}	5.57^{bc}	124.23^{bc}
LSD(5%)	17.23	6.46	16.14	3.90	106.33
CV (%)	29.59	27.77	26.59	31.77	33.62

Different letters within a column are significant at $P \leq 0.05$.

Effect of ALA on chlorophyll (Chl) content of *B. juncea* seedling under saline stress

A significant decline in Chl content was observed under saline stress (Fig. 1). Compared with the control (A_0S_0), total chlorophyll content decreased by 59.11%, in mustard seedlings treated with 12 dSm^{-1} salinity (A_0S_2). Application of ALA at both concentrations increased the chlorophyll content of leaves under any level of saline stress. The positive effects of ALA were more pronounced under salinity than under non-salinity conditions. Although higher concentration of ALA had better performance in increasing Chl in 8 dSm^{-1} salinity, higher concentration showed better role in Chl maintenance at 12 dSm^{-1}. In this connection, Chl in A_1S_2 treatment was higher than that in A_2S_2.

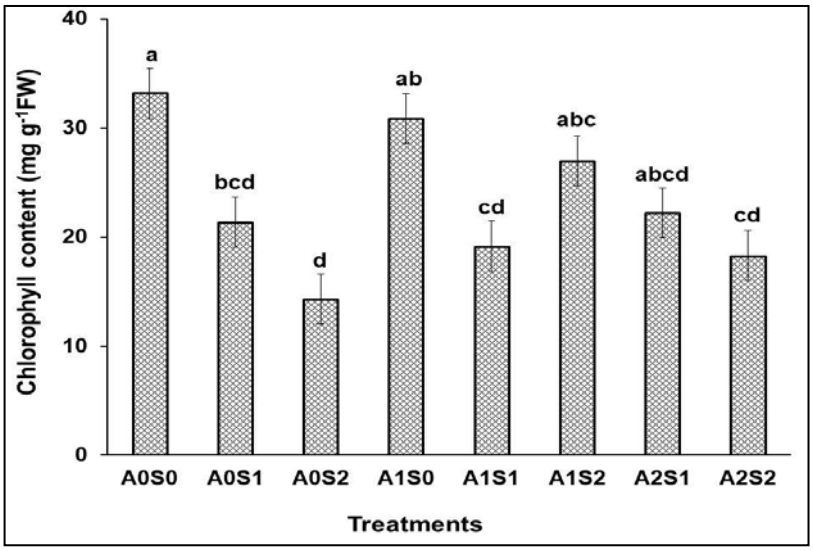

Fig 1. Effect of ALA on chlorophyll content on *B. juncea* seedling leaves under saline stress.

Chlorophyll content is used to indicate chloroplast development and is sensitive to abiotic stresses and very easy to degrade, and can lead to a reduction in photosynthetic capacity. In this study, saline stress can induce a serious decline in chlorophyll content and photosynthetic capacity, leading to a significant reduction in mustard seedling growth. ALA is a precursor of chlorophyll synthesis and forms proto-chlorophyllide in the proplastid and chlorophyll a through the reduction of light, and then chlorophyll a is oxidized to form chlorophyll b, preventing the decomposition of chlorophyllase. Moreover, ALA alleviates the harmful effects of salinity by regulating Chl synthesis pathway and leads to improve cucumber seedlings growth (Wu *et al.,* 2018). In the present study, chlorophyll content significantly increased after ALA treatment (Fig. 1). The increase in chlorophyll content is one of the reasons for the improved photosynthesis after ALA treatment. These findings suggest that ALA protects Chl biosynthesis under saline stress, thus improving mustard seedling growth (Fig. 1).

Effect of ALA on net photosynthesis (*Pn*), stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*), and transpiration rate (*Tr*) in *B. juncea* seedlings under saline stress

Saline stress (A_0S_1 and A_0S_2) caused significant reduction in *Pn* (53.45%), *Gs* (69.41%), *Ci* (42.08%), and *Tr* (71.15%) in mustard seedling compared to control (A_0S_0) (Table. 2). The low concentration of ALA (30 mgL^{-1}) showed better results than the high concentration of ALA (60 mgL^{-1}) in mustard seedling under salt stress. Treatment A_1S_2 increased the *Pn*, *Tr*, *Ci* and *Gs* of the leaves of

mustard seedlings by 72.86%, 130.8%, 35.72% and 124.87%, respectively, over the treatment without ALA (A_0S_2).

Table 2. Effect of 5-aminolevulinic acid (ALA) on saline-induced changes in net photosynthetic rate, stomatal conductance, intercellular CO_2-assimilation rate and transpiration rate in *B. juncea* seedling.

Treatments	Net photosynthetic rate (µmole CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (mmole H_2O $m^{-2}s^{-1}$)	Intercellular CO_2-assimilation rate (µmole CO_2 mol^{-1})	Transpiration rate (mmole H_2O $m^{-2}s^{-1}$)
A_0S_0	24.62^{a}	0.85^{a}	316.92^{a}	6.69^{a}
A_0S_1	15.76^{cd}	0.45^{bc}	214.12^{b-d}	3.76^{bc}
A_0S_2	11.46^{d}	0.26^{c}	183.57^{cd}	1.93^{c}
A_1S_0	23.59^{ab}	0.74^{ab}	293.87^{ab}	5.60^{ab}
A_1S_1	17.75^{a-d}	0.56^{a-c}	215.58^{b-d}	4.03^{bc}
A_1S_2	19.81^{a-c}	0.60^{ab}	249.15^{a-c}	4.34^{a-c}
A_2S_1	16.16^{b-d}	0.46^{bc}	155.51^{d}	5.27^{ab}
A_2S_2	13.85^{cd}	0.45^{bc}	192.48^{cd}	5.37^{ab}
LSD (5%)	7.54	0.31	81.51	2.54
CV	24.75	33.82	21.01	32.18

Different letters within a column are significant at $P \leq 0.05$.

Photosynthesis is the basis of plant growth and development. Under salinity stress, *Gs* and *Tr* decrease remarkably. This decrease causes a reduction in shoot transpiration. Thus, the leaf stomata of mustard seedlings have closed. The Na^+ concentration from leaves may be limited by reducing the leaf *Tr* because the amount of Na^+ transported to plants is in proportion to the leaf *Tr*, with a relatively reduced absorption of toxic ions (Contreras-Cornejo *et al.*, 2014). The decrease in the leaf *Tr* of mustard seedlings under short-term salinity stress is an adaptive response. This phenomenon belongs to a mechanism of non-halophytes to prevent Na^+ stress and to adapt to osmotic stress. In this study, treatment 12 dS^{-1} salinity was reduced the *Ci*, *Tr*, *Pn*, and *Gs* and ALA application could also improve them (Table 2). Therefore, ALA possibly improves *Pn* of mustard seedlings under salinity stress, and this role is likely related to the enhanced *Gs* and increased C_i. However, foliar application of ALA enhanced the photosynthetic parameters by improving saline tolerance; as reported by Ye et al. (2016) in peach. These findings suggest that saline stress decreased or decline in photosynthetic capacity (*Pn, Gs, Ci,* and *Tr*) in

mustard seedlings, but that capacity was significantly enhanced by foliar ALA application (Table 2).

Effects of ALA on Na^+ and K^+ accumulation in response to salinity stress

Sodium ion content was significantly increased by 105.99% in mustard leaves in A_0S_2, as compared to control (A_0S_0). Unlike Na^+, K^+ decreased by 24.27% and 19.04% in A_0S_1 and A_0S_2, respectively, as compared to A_0S_0. The Na^+ content and Na^+/K^+ ratio in leaves increased significantly in both salinity levels (A_0S_1 and A_0S_2) as compared to A_0S_0 (Table. 3). Treatments containing ALA reduced Na^+ content and Na^+/K^+ ratio compared to treatments without ALA. The K^+ content decreased in salinity, and ALA containing salinity treatments had comparatively higher K^+. However, no significant differences were observed between low and high ALA containing saline treatments (Table. 3).

Table 3. Effect of 5-aminolevulinic acid (ALA) on saline-induced changes in Na^+, K^+ content and Na^+/K^+ ratio on *B. juncea* seedling.

Treatments	Na^+ content (ppm)	K^+ content (ppm)	Na^+/K^+ ratio
A_0S_0	1885^{bc}	2843^{ab}	0.66^{b}
A_0S_1	2956^{a-c}	2593^{a-c}	1.14^{b}
A_0S_2	3883^{a}	2153^{d}	1.81^{a}
A_1S_0	1840^{c}	2903^{a}	0.64^{b}
A_1S_1	2988^{ab}	2563^{a-d}	1.19^{ab}
A_1S_2	2531^{bc}	2351^{cd}	1.18^{ab}
A_2S_1	2556^{bc}	2460^{b-d}	1.11^{b}
A_2S_2	2766^{a-c}	2593^{a-c}	1.09^{b}
LSD (5%)	1145	417.54	0.64
CV	25.13	9.58	33.92

Different letters within a column are significant at $P \leq 0.05$.

Salinity stress is characterized by Na^+ toxicity and ion imbalance, which is caused by their placement of K^+ by Na^+ in plants sensitive to salinity stress. Salinity increases the content of Na^+ in mustard plant tissue but the content of K^+ reduces. The maintenance of low Na^+/K^+ ratio in plants is one of the major characteristics of salinity tolerance and the ratio is equal or lower than 0.6 as a considered optimal for maintaining normal metabolic processes in plants. Foliar application of ALA with salinity alleviated the ionic (Na^+ and K^+) balance and promoted its growth of many plant species (Akram *et al.*, 2011;

Naeem *et al.,* 2012 and Yang *et al.,* 2014). In this study, salinity stress increased Na^+ content and decreased K^+ content, leading to increases in Na^+/K^+ ratio approaching 1.0 in mustard seedling. Moreover, foliar application of ALA reduced Na^+ content in leaves, although it had no effects on K^+, resulting in lower Na^+/K^+ ratio. Our results suggested that ALA did not affect K^+ accumulation, but could suppress root uptake of Na^+ or transport of Na^+ from roots to leaves, resulting in lower Na^+/K^+ ratio and there by mitigating Na^+ toxicity in mustard leaves.

Effect of ALA on proline, MDA, $O_2^{\bullet-}$ and H_2O_2 of *B. juncea* seedlings under saline stress

As compare to control (A_0S_0), Pro and MDA contents increased significantly under saline treatments (A_0S_1) and (A_0S_2) in mustard seedling, being higher in A_0S_2 (Table 4). Moreover, foliar application of ALA increased both Pro and MDA contents in saline treated seedlings, low concentration being more effective. As compare to A_0S_2, A_1S_2 increased Pro by 243.47% and 172.17%, and MDA by 127. 9% and 105.31%, respectively. Further, we investigated the $O_2^{\bullet-}$ and H_2O_2 formation in mustard seedling leaves where the concentration of both ROS increased significantly in saline stresses, being gradually higher with increasing salinity level (Table 4). It was important that ALA containing salinity treatments (A_1S_1, A_2S_1, A_1S_2, A_2S_2) reduced the formation of both ROS that the saline treatments with ALA. It was also remarkable that low ALA containing treatments (A_1S_1 and A_1S_2) were more effective in reducing the ROS the treatments with higher ALA (A_2S_1 and A_2S_2). Saline stress at 12 dS^{-1} increased the levels of H_2O_2 and $O_2^{\bullet-}$, by 165.50% and 159.9%, respectively, compared with those in the control (Table 4). Moreover, treatment with ALA (30 mgL^{-1} and 60 mgL^{-1}) and 12 dS^{-1} salinity decreased the H_2O_2 and $O_2^{\bullet-}$ contents by 10.39%, 16.15% and 12.54%, 15.82%, respectively. Meanwhile, ALA treatments did not significant change the $O_2^{\bullet-}$ and H_2O_2 content in mustard seedlings.

The Pro content increases under salinity stress. Hence, the absorption and accumulation of ions in crops are affected, their cytosolic concentration is reduced, and cellular water potential is increased. ALA may increase cellular fluid concentration under salinity stress, reduce cellular water potential, and enhance water absorption in cells by increasing proline content. Our results show that foliar application of ALA enhanced proline accumulation in the leaves under salinity stress (Table 4), as also reported by Naeem *et al.* (2012) and Ye *et al.* (2016). Salinity stress may also induce the peroxidation of membrane lipids and MDA content and membrane permeability are important

indicators to evaluate membrane lipid peroxidation and plasma lemma damage. The salinity stress treatment induced a significant increase in MDA content in mustard seedlings (Table 4). This result is significantly higher than that in normally grown plants. Therefore, the plasma membrane of plant cells undergoes peroxidation and exhibits physiological disruption. Seedlings treated with 30 mgL^{-1} ALA and 12 dS^{-1} salinity showed reduces in MDA content, which suggests that ALA treatment could relieve salinity-induced inhibition of MDA content in mustard seedlings. However, response to foliar ALA application varies among different plants, stage of plant development, timing, and applied concentration (Ye *et al.*, 2016). Salinity enhanced the level of H_2O_2 and $O_2^{\cdot -}$ generation whereas ALA application helped the salinity-treated plants to detoxify both ROS by manipulating the antioxidant enzyme activities as reported by Nishihara *et al.* (2003) in *Spinacia oleracea* leaves. However, the production of H_2O_2 and $O_2^{\cdot -}$ generation has been shown to depend on the intensity of stress and ALA at different concentrations reduced the $O_2^{\cdot -}$ generation and H_2O_2 contents in *B. napus* leaves under salinity conditions (Naeem *et al.*, 2011). In the present study, the response of H_2O_2 and $O_2^{\cdot -}$ generation saline stress with ALA was similar to the previous results.

Table. 4. Effect of 5-aminolevulinic acid (ALA) on saline-induced changes in proline (Pro) and melondialdehyde (MDA) content, superoxide ($O_2^{\bullet -}$) generation and hydrogen peroxide (H_2O_2) content in *B. juncea* seedlings.

Treatments	Pro content (μmol g^{-1} FW)	MDA content (nmol g^{-1} FW)	$O_2^{\bullet -}$ generation ($nmol^{-1}g^{-1}min$ FW)	H_2O_2content (μmol g^{-1} FW)
A_0S_0	5.87^{c}	14.59^{c}	0.84^{e}	7.15^{b}
A_0S_1	11.01^{bc}	23.48^{bc}	1.48^{d}	12.18^{ab}
A_0S_2	20.16^{a}	33.25^{ab}	2.23^{a}	18.58^{a}
A_1S_0	5.58^{c}	13.78^{c}	0.89^{e}	7.03^{b}
A_1S_1	13.83^{a-c}	22.48^{bc}	2.02^{b}	13.58^{ab}
A_1S_2	20.15^{a}	37.93^{a}	1.92^{bc}	16.51^{a}
A_2S_1	16.91^{ab}	33.61^{ab}	2.05^{b}	15.64^{a}
A_2S_2	14.03^{a-c}	32.25^{ab}	1.85^{c}	11.77^{ab}
LSD(5%)	8.73	12.77	0.79	6.88
CV	38.15	28.63	27.77	31.56

Different letters within a column are significant at $P \leq 0.05$.

Effect of ALA on the antioxidant enzyme activities of *B. juncea* seedling under saline stress

The antioxidant enzyme activities of SOD, APX, CAT, and POD in mustard leaves treated with salinity stress and ALA are shown in Table 5. The activities increased gradually and significantly with increasing salinity level. The highest salinity level (A_0S_2) increased the activities of SOD, APX, CAT, and POD by 153.2%, 101.98%, 83.44%, and 77.20%, respectively, as compare to control (A_0S_0). The ALA treated salinity treatments further increased the activities compared to respected non-treated salinity treatments. In most of the cases, lower concentration ALA added salinity treatments (A_1S_1 and A_1S_2) showed better performance in increasing the activities as compare to higher ALA added salinity treatments (A_2S_1 and A_2S_2).

Table. 5. Effect of ALA on saline-induced changes in SOD, APX, CAT and POD activities in *B. juncea* seedling.

Treatments	SOD activity (Unit $min^{-1}mg^{-1}$ protein)	APX activity (µmol $min^{-1}mg^{-1}$ protein)	CAT activity (µmol $min^{-1}mg^{-1}$ protein)	POD activity (µmol $min^{-1}mg^{-1}$ protein)
A0S0	58.73^{c}	129.47^{c}	82.74^{b}	181.31^{d}
A0S1	86.49^{c}	204.61^{bc}	135.65^{a}	284.91^{bc}
A0S2	148.68^{a}	261.39^{ab}	151.78^{a}	321.31^{a-c}
A1S0	61.25^{c}	135.68^{c}	81.94^{b}	178.15^{d}
A1S1	97.30^{bc}	197.51^{bc}	126.14^{ab}	268.16^{cd}
A1S2	137.68^{ab}	290.24^{a}	163.72^{a}	367.39^{ab}
A2S1	153.58^{a}	295.99^{a}	168.61^{a}	385.50^{a}
A2S2	144.59^{ab}	274.95^{ab}	150.85^{a}	380.90^{ab}
LSD (5%)	50.73	82.68	46.52	97.69
CV	26.82	21.70	20.58	19.38

Different letters within a column are significant at $P \leq 0.05$.

Salinity stress may also promote the establishment of plant defense systems. Antioxidant is another protective enzyme in plant defense systems. SOD may catalyze the disproportionation reaction in vivo and thus transforms superoxide radical into O_2 and H_2O_2. As a result, plants are protected against damage by reactive oxygen under salinity stress. In this study, the antioxidant activity in the leaves of mustard seedlings is increased at 12 dS^{-1} salinity (Table 5). An increase

in the antioxidant activity may maintain the metabolic balance of ROS in peach seedlings, protect the integrity of membrane structure, and avoid damage induced by salinity. The regulatory mechanism involves ferroheme-containing POD as a prosthetic group and ALA as a precursor of porphyrin heme biosynthesis. ALA treatment promotes the synthesis of ferroheme and increases the activity of POD as a prosthetic group; thus, resistance to oxidative stress is enhanced. The present research, application of 30 mgL^{-1} ALA was enhanced the CAT, SOD, APX, and POD activities in the leaves of mustard seedlings under salinity stress (Table 5). The CAT and APX activities play an important role in scavenging H_2O_2 to form H_2O and O_2 within the glutathione-ascorbate cycle, in protecting a plant against oxidative stress. The previous study suggested that CAT, POD, and APX contain a heme prosthetic group, while ALA is a key precursor of heme biosynthesis (Zhang *et al.*, 2015), which might be the reason that ALA-treated seedlings showed increased antioxidant enzyme activity (Table 5), reducing the overproduction of ROS and MDA (Table 4) in mustard seedling under saline stress.

Conclusion

Foliar application of ALA was effective for mitigating salinity damages in *B. juncea* seedling. The effects of ALA at low concentration in *B. juncea* were associated with the suppression of Na^+ toxicity and enhanced plant growth, promote the photosynthesis, accumulation of photosynthetic products, ion exchange, proline, chlorophyll content and enzymatic antioxidant activity, and inhibit the accumulation of lipid peroxidation product, H_2O_2, $O_2^{\bullet-}$, preventing cellular damages. Results suggest that ALA has the potential to overcome the adverse effects of salinity in *B. juncea.* Further research at molecular levels may be necessary to identify molecular and metabolic pathways conferring ALA effects on stress mitigation in *B. juncea* and other plant species. In addition, field tests should be conducted to further examining the feasibility and effectiveness of ALA to promote plant tolerance to salinity under natural environmental conditions.

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