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SALICYLIC ACID ALLEVIATES SALT STRESS IN SOYBEAN BY IMPROVING ANTI-OXIDANT CAPACITY AND GROWTH PERFORMANCE

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Abstract

The paper investigated the effects of salicylic acid (SA, 0, 0.5, and 0.75 mM) on soybean (*Glycine max*) development, antioxidant enzyme activity, and biochemical characteristics under salt stress. The results showed that, salinity exposure (0, 25, 75, and 125 mM NaCl) inhibited soybean growth by lowering the fresh weight of the shoot and root, inhibiting leaf development, and triggering necrosis on old leaves. With 125 mM NaCl level, lipid peroxidation or hydrogen peroxide (H2O2) production rose by 3.37 and 2.54, respectively, whereas membrane stability decreased, which was alleviated by the addition of SA. Compared with the control, SA (0.5 mM) considerably boosted growth. Salinity (125 mM NaCl) stress increased proline accumulation by 4.63 times, carbohydrates by 39.61 percent, free amino acids via 9.44 percent, and protein content via 7.91 percent, all of which were further promoted by SA, indicating a greater stress adaption. Application of SA increased the activity of antioxidant enzymes including SOD, CAT, and APX by 1.76, 2.25, and 2.22 times, respectively, resulting in improved reactive oxygen species removal and protection from oxidative stress. Furthermore, excessive Na^+ uptake in salinity-stressed plants inhibited K^+ absorption and triggered leaf necrosis, while application of SA significantly reduced the damage. These results suggested salt stress has a negative impact on soybean growth and development and supplementing with a suitable dose of SA alleviated the detrimental effects by modulating osmolyte levels, antioxidant enzyme activity, and critical element intake.

Key words: Soybean, Salicylic acid, Antioxidant enzymes, Necrosis, Lipid peroxidation, Salinity, Proline

Introduction

Salt stress is one of the main abiotic stress conditions that limit plant productivity and growth (Allakhverdiev *et al*., 2000). It affects practically all plant activities by altering numerous biochemical and physiological responses (Iqbal *et al*., 2006). Under salt stress, significant decreases in growth, including decreased leaf length, leaf area, and shoot and root dry weight were observed (Saleh, 2012). Extraordinary salt stress can also limit photosynthesis by reducing green pigment content and promoting stomatal closure as well as oxidative stress, culminating in the age group of reactive oxygen species (ROS) (He *et al*., 2014; Nazar *et al*., 2011), which will produce electrolyte leakage, increase membrane lipid peroxidation, and harm the chloroplast, slowing photochemical processes and reducing photosynthesis (Gunes *et al*., 2007; Steduto *et al*., 2000). Plants have developed an excellent mechanism by promoting enzymatic antioxidants to mitigate the deleterious effects of this ROS.

Salicylic acid (SA) is an essential signalling molecule that affects a diversity of physiological as well as biochemical activities in plants, and the tolerance to abiotic or biotic stresses (Syeed *et al*., 2011). There has been research on the function of SA in plant tolerance to abiotic stimuli such ozone, heat,UV-B, heavy metals, osmotic stress, and heat (Wang *et al*., 2010). It has been reported that SA treatment relieved the negative possessions of salt stress in soybean by improving plant photosynthesis and development, as well as increasing the antioxidant status (Khan *et al*., 2013). Exogenous SA, on the other hand, improves salt tolerance depending on genotype and SA level. Exogenous SA, for example, was shown to prevent salt stress-induced development suppression in a salt-tolerant soybean genotype, however not in a salt-complex cultivar (Arfan *et al*., 2007). As a result, more investigation is required to determine if the efficiency of SA in reducing salt stress is dependent on the plant type.

Soybean (*Glycine max*) originated in China, it is a main feed and food crop in several nations, particularly China, and has finally gotten a lot of care from Chinese farmers due to the high need. Soybean farming, similar further cereal crops, is threatened by rising soil salinity, particularly in China south areas. Discovery effective technology to manage the decrease in soybean output due to salt stress is thus critical for the nation's food safety. The aim of this study is to look into the possible involvement of SA in reducing salt toxicity and increasing soybean production under saline circumstances, which will help to understand the salt tolerance mechanism of soybean and give a guide to promoting soybean cultivation in the world.

Materials and Methods

Sterilized soybean (*Glycine max*) seeds were sowed in 30 cm diameter plastic pots comprising clay soil and kept in a greenhouse underneath natural circumstances for growth. Until the development of the fourth true leaves, all pots were watered by double distilled water (DDW). After that, the plants were exposed to numerous salt levels (0, 25, 75, and 125 mM NaCl), and varying amounts of salicylic acid (SA) (0, 0.5 and 0.75 mM). SA was sprayed exogenously at a rate of 20 ml per pot, whereas the switch plants were merely given DDW in the same amount. Plants were removed and weighed for dry and fresh weight after 27 days of growth. The plant material was maintained in the oven for 24 hours at 105°C for dry weight.

Determination of carbs, free amino acids, total protein, and free proline: The proline content was determined according to Bates *et al*., (1973) method. The free amino acid content was measured according to Moore and Stein's approach (Moore & Stein, 1948). The carbohydrate content was determined using anthrone-sulfuric acid technique (Schlegel, 1956). The total protein was calculated using Lowry's method (Lowry *et al.*, 1951).

Determination of hydrogen peroxide (H_2O_2), electrolyte leakage (EL) and malondialdehyde (MDA): H_2O_2 was measured, according to the method of Mukherjee & Choudhuri (1983), while MDA was determined according to the method of Rao & Sresty (2000). The EL was measured using the methodology of Dionisio-Sese & Tobita (1998).

Assay of antioxidants: A 5 mg leaf sample was homogenised in 100 mM Tris-HCl (pH 7.5) comprising DTT (5 mM), EDTA (1 mM), MgCl2 (10 mM), PVP40 (1.5 percent), PMSF (1 mM), and aproptinin (1 g mL-1) magnesium acetate (5 mM), and aproptinin (1 g mL-1). After crushing, the material was passed throughout a thin cloth as well as centrifuged for 15 minutes at 4°C at 10,000 rpm. The acquired material was employed as an enzyme basis. The activities of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase(APX) , and catalase (CAT, EC 1.11.1.6) was determined using the reported procedures (van Rossum *et al*., 1997; Aebi, 1984 ; Nakano & Asada, 1981).

For the purpose of Na+ and K+ in ground plant material, 200 mg of dried herbal material (roots & shoots) was combined with 20 ml of focused H_2SO_4 as well as perchloric acid on a heated plate for 2 hours. Volume makeup was done using distilled water after the result was filtered via filter paper volumetric flasks into 50 mL. A flame photometer was used to test the levels of K+ and Na+.

Statistical analysis: SPSS 23.0 was used to do a one-way ANOVA here on data. Tukey's post-hoc test was used to separate means in homogenous groupings in multiple comparisons.

Results and Discussion

Effects of salt in addition salicylic acid on dry and fresh mass: In comparison to control plants, salinity stress (125 mM) reduced shoot FW via 44.79 percent and DW via 49.13 percent. As SA (0.50 mM) was added to NaCl (125 mM) preserved plants, the DW and FW of the shoots increased by 30.68 and 32.88 percent. However, compared with the control, SA (0.75 mM) reduced shoot DW and FW (Fig. 1A-B).

Ahmad *et al*., (2015) found that NaCl stress reduced plant biomass output in mustard, mulberry, chickpea, and broad bean (Ahmad *et al*., 2014; Rasool *et al*., 2013; Azooz *et al*., 2011). Under extreme salt stress, Mohamed & Shaddad (2013) found that salinity had a deleterious influence on soybean growth and production. Because of enhanced sodium ion level or osmotic pressure inside leaf cell cytoplasm, high salt concentrations inhibited shoot development. Sodium ions might be a toxicity factor causing lower dry weights in shoots (Pitann *et al*., 2013; Orsini *et al*., 2012). In this investigation, SA supplementation increased dry and fresh weight, and the findings are consistent with those of Iqbal *et al*., (2006) in soybean cultivars. Gunes, (2007) also found that treating maize plants with SA increased their dry weight associated to untreated plants cultivated under NaCl stress. Supplementation of SA improved the development and biomass output of *Gossypium Hirsutum* further down NaCl stress, according to Muhammad *et al*., (2022).

H_2O_2, MDA concentration, and EL: Effects of varying salinity levels as well as exogenous SA application: As indicated in Fig. 2A-C, salt stress increased H_2O_2, MDA concentration, and EL at all stress levels. In contrast to control plants, H_2O_2, MDA level, and EL were 2.54, 3.37, and 2.00 times higher in NaCl (125 mM) preserved plants. In evaluation to NaCl alone treated plants, adding SA (0.50 mM) to NaCl 125 mM plants reduces H_2O_2 material by 3.43 times, MDA material via 1.80 times, and EL via 2.59 times. H_2O_2, MDA, and EL levels are all higher in SA (0.75 mM) treated plants than in NaCl plants.

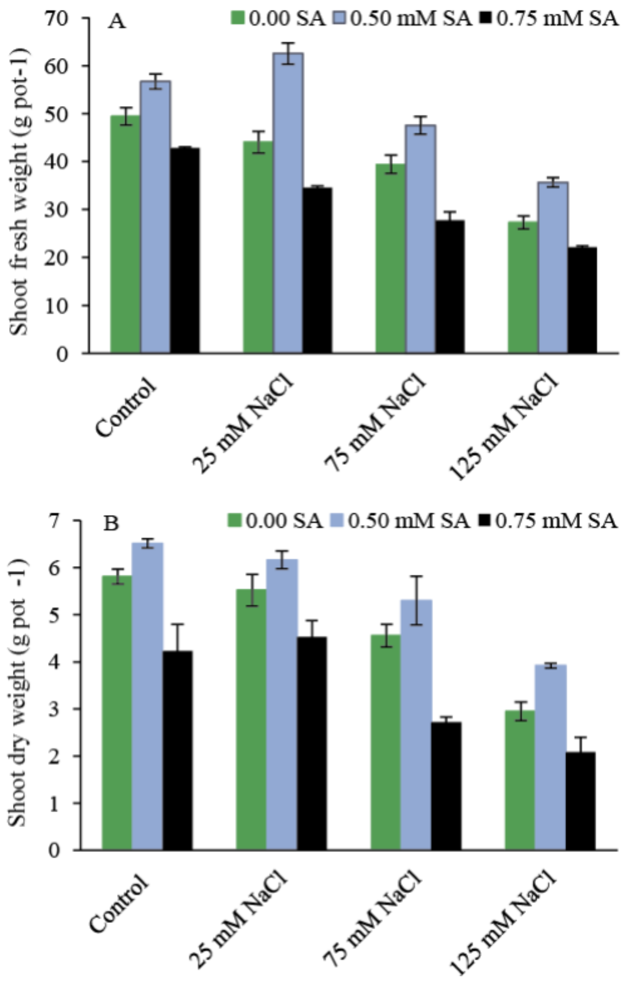
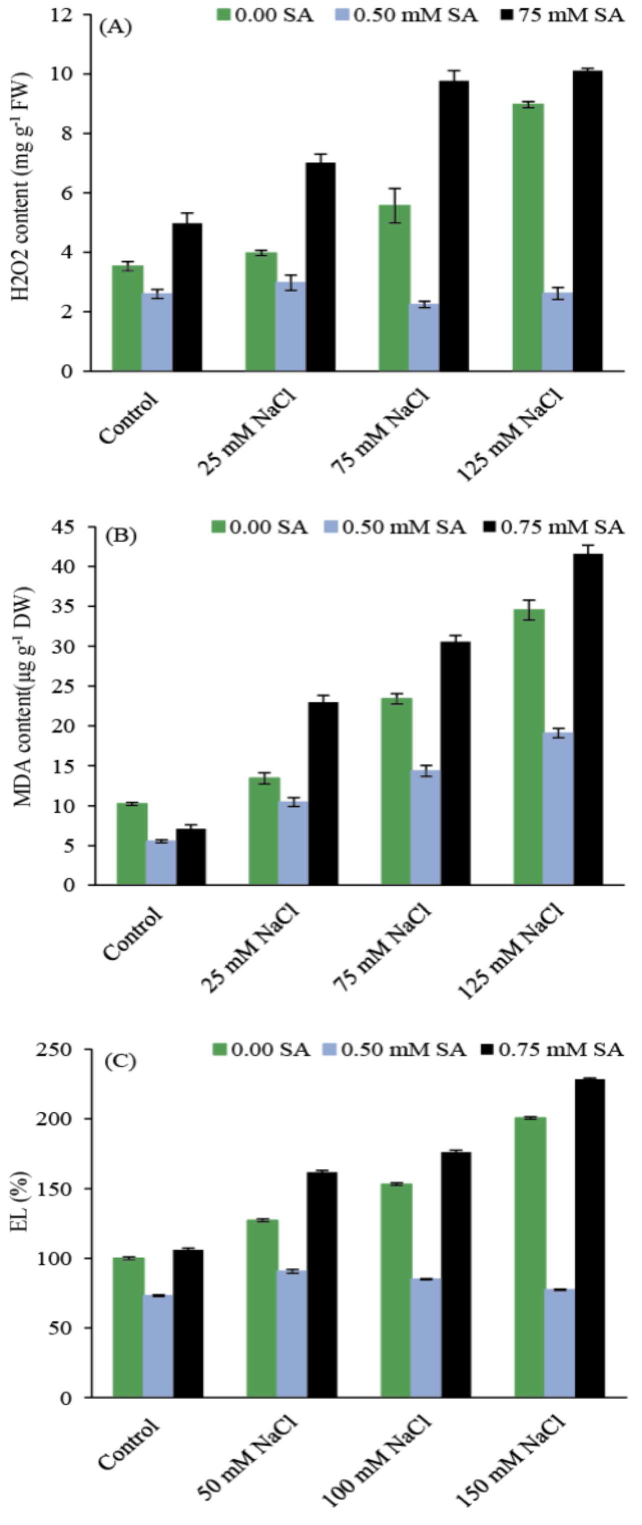


Fig. 1. Salicylic acid and varied amounts of NaCl interact to affect (A) fresh and (B) dry weight of soybean plant shoots (g pot1). The values characterize the M and SD of three separate replications (n = 5).



Fig, 2. Salicylic acid and varied amounts of NaCl interact to affect the (A) %. In soybean plants, lipid peroxidation, electrolyte leakage, and hydrogen peroxide are all present. The values signify the M & SD of three separate replications (n = 5).

The findings of Ahmad *et al*., (2015) in *Brassica juncea* are supported by the elevated levels of MDA content, H_2O_2, and EL. Zhang *et al*., (2014) found that NaCl stress enhanced H_2O_2 accumulation in cotton seedlings, and Giannakoula & Ilias (2013) found that NaCl stress enhanced H_2O_2 deposition in tomato seedlings. The increased H_2O_2 level might be associated to a lessening in H_2O_2 scavenging activity (Senadheera *et al*., 2012). The levels of MDA and H_2O_2 in plants are thought to be markers of the severity of oxidative stress (Ahanger, 2017a and b). Membrane damage is caused by salinity through membrane lipid peroxidation (Mishra & Choudhuri, 1999), and our findings revealed a positive relationship among membrane permeability and MDA content. Improved H_2O_2 production speeds up the Haber-Weiss process, causing more membrane damage. Under salt stress, Ahmad *et al*., (2014) established that MDA levels in mulberry were raised, resulting in improved electrolyte leakage. In Okra and tomato, Saleem *et al*., (2011) and Li (2009) both demonstrated a synergistic impact of NaCl and MDA buildup. In the current study, SA supplementation reduced H_2O_2, MDA buildup, in addition to electrolyte leakage. Below NaCl stress, SA serves as an antioxidant and aids in the scavenging of ROS such as H_2O_2 (Ahmad *et al*., 2015). By quenching ROS, antioxidants including CAT, APX, and SOD aid to reduce the buildup of H_2O_2 and MDA, which reduces membrane peroxidation and electrolyte leakage (Ahmad *et al*., 2015). SA, according to Shen *et al*., (2014), served as a signalling molecule in plants, activating several defence systems. MDA levels in soybean reduced considerably after treatment with salicylic acid, indicating that SA can help soybean cope with stressful situations by improving membrane integrity.

Figure 3 shows the results for free amino acids, proline, entire carbs, and protein material. In comparison to control plants, NaCl (125 mM) increased proline material by 4.63 times. With 0.50 mM as well as 0.75 mM SA, the proline material in NaCl plants was increased by 1.81 and 5.12 times, respectively (Fig. 3A). When compared to control, the greater NaCl concentration (125 mM) increased free amino acids, whole carbohydrate, as well as protein content via 9.44 percent, 39.61 percent, and 7.91 percent, respectively (Fig. 3B-D). Over NaCl alone treated plants, SA (0.50 mM) increased free amino acid concentration by 19.87 percent, total carbohydrate level by 38.72 percent, and protein content by 22.51 percent. Proline accumulation has been seen in *D. superbus, Brassica juncea, and Lens culinaris* during salt stress (Ma *et al*., 2017; Misra & Saxena, 2009; Ahmad *et al*., 2015). Plants that have more proline contribute to osmotic adjustment and membrane integrity (Misra & Saxena, 2009). Proline has antioxidant properties and aids in the quenching of ROS, therefore protecting cells from oxidative damage (Jogaiah *et al*., 2013; Ahmad *et al*., 2010). According to Aggarwal *et al*., (2012), proline had a function in energy storage below NaCl stress. The present study's findings on increased concentrations of free amino acids are compatible with those of Jaleel *et al*., (2008), who found a variable buildup of amino acid concentrations through varying NaCl stress concentrations. SA treatment reduced the detrimental possessions of NaCl toxicity happening amino acid concentrations in this investigation. According to these findings, Palma *et al*. (2013) found that spraying SA increased the movement of such photosynthetic machinery and enzymes in beans. It's been proposed that when SA concentrations rise, so do amino acid levels in soybean (Khan *et al*., 2013). Yin *et al*., (2010) found increased carbohydrate content in *Solanum lycopersicum* during salt stress. NaCl increased carbohydrate content in *Phragmites australis*, according to Hartzendorf & Rolletschek (2001). Carbohydrate metabolism is extremely susceptible to salt stress (Oliver *et al*., 1998).

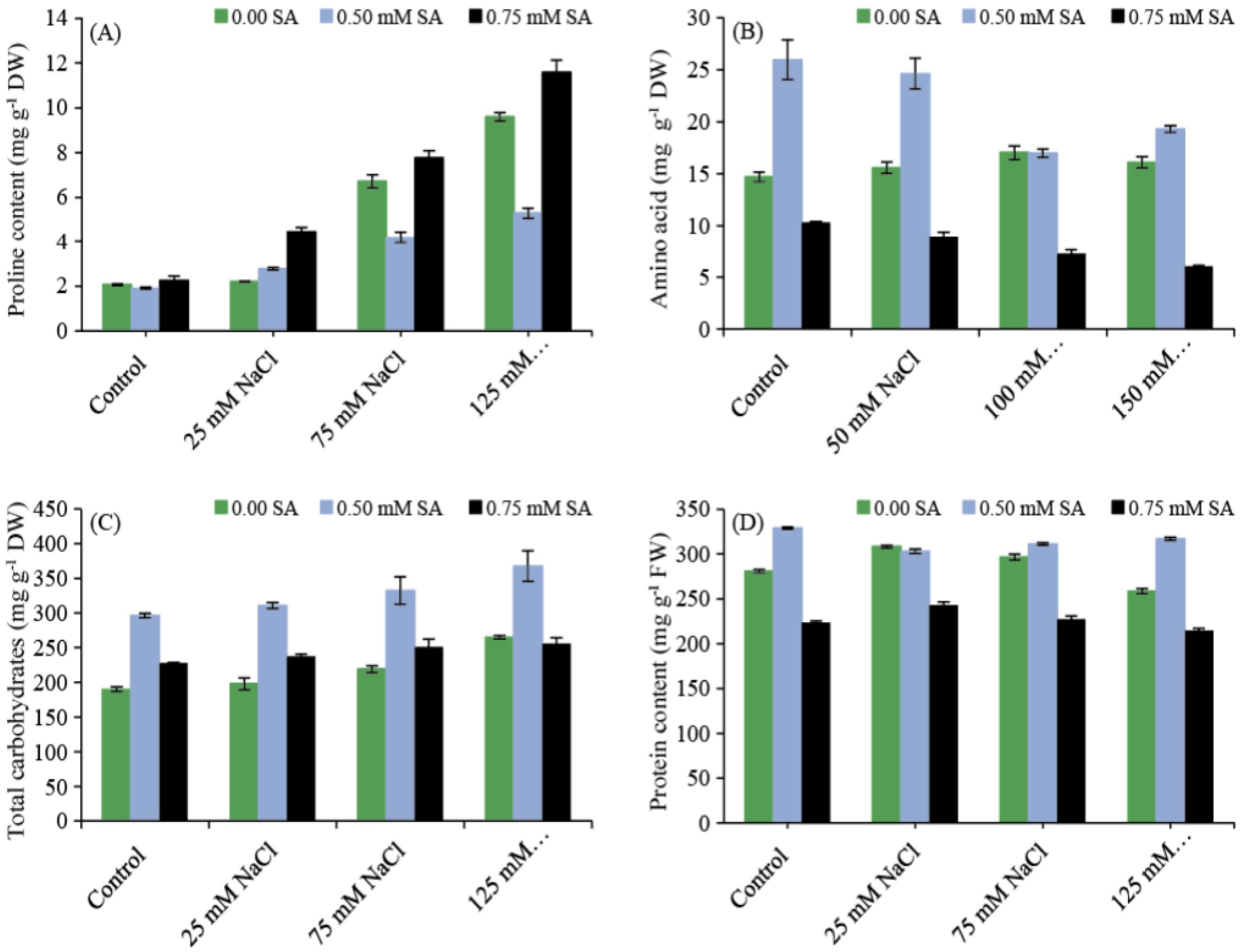


Fig. 3. Shows the interaction of salicylic acid in addition to various amounts of sodium chloride on proline (A), total carbs (C), total protein (D) and amino acids (B) in soybean plants. The values represent the means and standard deviations of three separate replications (n = 5).

In the current investigation, SA supplementation dramatically increased glucose storage in both control and salt challenged plants. In the case of soybean, our findings are reliable with those of Agamy *et al*., (2013). Increased glucose levels under salt stress, according to Naureen & Naqvi (2010), might be useful characteristics for determining salt-tolerant soybean plants. This rise in whole carbohydrate concentrations is an osmoregulation process (Siringam *et al*., 2012; Shaddad *et al*., 1990) that protects plants from oxidative stress while also preserving protein and membrane structures (Hajihashemi *et al*., 2006). In the current investigation, soluble proteins exhibited an increase at lesser doses of NaCl, but a loss at greater amounts (125 mM). The use of SA, on the other hand, increased protein buildup. Proteins are thought to show a significant part in osmotic change (Ashraf, 2004). Accumulated proteins operate as a nitrogen storage method (Ahmad *et al*., 2015).

Na+ and K+ levels in the shoots and roots, as well as leaf necrosis: NaCl stress (125 mM) increased shoot Na+ via 182.60 percent and root Na+ via 1241.66 percent, respectively, as associated with control plants. In comparison to NaCl-only treated plants, 0.50 mM SA supplementation reduced Na+ accumulation by 26.15 percent in the shoot and 14.90 percent in the root (Table 1). In comparison to control, 125 mM NaCl (125 mM) lowered shoot K+ by 3.60 percent and root K+ by 22.66 percent (Table 1). When compared to NaCl-only treated plants, SA (0.50 mM) increased root and shoot K+ by 8.41 and 167.24 percent, correspondingly. Under NaCl stress, SA (0.75 mM) reduced K+ buildup in both the shoot and the root.

Excess Na+ is hazardous to plant development, whereas K+ is essential for plant growth control due to its participation in processes such as enzyme activation, protein synthesis, and osmoregulation, among others (Ahanger, 2017; 2017a). Greater K+ accumulation protects growth below stressful situations by reducing oxidative harm to membranes, and SA treatment increased K+ uptake and accumulation in the current investigation, most likely via modifying the ion carrying proteins are present in its approval. SA reduced excessive Na+ buildup and facilitated its exclusion and division into less sensitive tissues such as the apoplast as well as vacuole, resulting in oxidative damage mitigation (Syeed *et al*., 2011). SA defends the photosynthetic machinery from injury by mediating the avoidance of excessive Na+ buildup in shoot tissues (Syeed *et al*., 2011).

Table 1. shows the interaction of salicylic acid in addition to various amounts of NaCl on the concentrations of K+ and Na+ ions in the roots and shoots of soybean plants.

Parameters / Treatments		Shoot Na^+ Conc.	Root Na^+ Conc.	Shoot K^+ Conc.	Root K^+ Conc.
		(mmol mg^{-1})			
0.00 mM SA	Control	0.23 ± 0.01e	0.12 ± 0.03c	0.111 ± 0.001c	0.075 ± 0.002b
	25 mM NaCl	0.30 ± 0.04c	0.93 ± 0.01c	0.115 ± 0.001c	0.081 ± 0.005ab
	75 mM NaCl	0.38 ± 0.10b	1.20 ± 0.06b	0.114 ± 0.004ab	0.079 ± 0.001b
	125 mM NaCl	0.65 ± 0.01a	1.61 ± 0.02a	0.107 ± 0.001bc	0.058 ± 0.002c
0.50 mM SA	Control	0.23 ± 0.01de	0.14 ± 0.04d	0.117 ± 0.004a	0.079 ± 0.007d
	25 mM NaCl	0.29 ± 0.01cde	0.37 ± 0.08d	0.121 ± 0.003ab	0.113 ± 0.005bc
	75 mM NaCl	0.31 ± 0.01cd	1.24 ± 0.01b	0.108 ± 0.004cd	0.147 ± 0.003a
	125 mM NaCl	0.48 ± 0.05b	1.37 ± 0.15ab	0.116 ± 0.002bc	0.123 ± 0.004b
0.75 mM SA	Control	0.20 ± 0.03e	0.12 ± 0.03c	0.109 ± 0.00bc	0.118 ± 0.002b
	25 mM NaCl	0.33 ± 0.02c	0.63 ± 0.04c	0.110 ± 0.01bc	0.091 ± 0.005ab
	75 mM NaCl	0.56 ± 0.10b	0.99 ± 0.05b	0.107 ± 0.001bc	0.103 ± 0.002cd
	125 mM NaCl	0.77 ± 0.02a	1.59 ± 0.03a	0.096 ± 0.002c	0.155 ± 0.001a

Values are mean ± 3 SE. Bars with different letters showed significant difference at $p \leq 0.05$

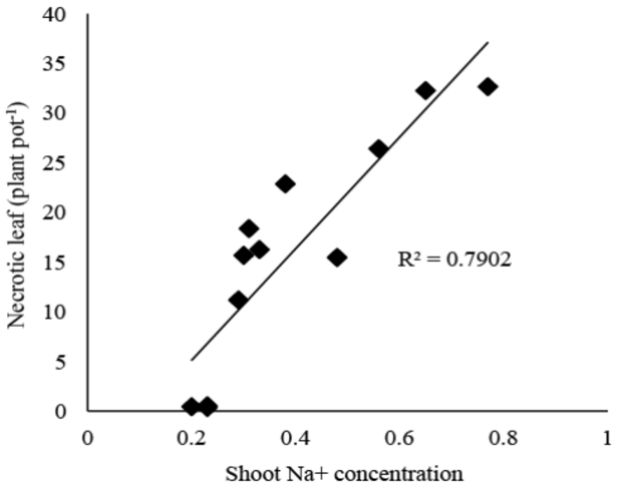


Fig. 4. Shows the interaction of salicylic acid and various NaCl concentrations on the relationship among shoot Na+ level and necrotic leaf for every plant in soybean plants. The values represent the resources and standard deviations of three separate replications (n = 5).

As evidenced by the findings of this study, a low Na+ content in shoots combined with a reduction in the emergence of indications of foliage issues can be regarded appropriate metrics for identifying salt resistance (Eker *et al*., 2006). In soybean, buildup of low Na+ levels inside the shoot donated significantly to increasing the dry and fresh masses of salt tolerant cultivars, according to Saqib *et al*., (2005). In the current work, exogenous SA administration drastically reduced Na+ concentrations in the leaves to the lowest level achievable, allowing it to play its positive effect. SA had a favourable effect on the number of necrotic leaves, presumably due to a decrease in Na+ absorption (Fig. 4 and Table 1). The growth of particular necrotic signs on the old foliage was caused by a higher salt treatment (125 mM NaCl). During controlled conditions, SA treatment at 0.5 mM protected plants against salinity-induced necrosis as well as caused in the formation of better leaves contrasted to control plants. However, a considerable growth in the percentage of necrotic leaves was detected at 0.75 mM SA, indicating that the doses are hazardous to the soybean cultivar. Moreover, salt stress raised the amount of necrotic leaves more than just the non-saline-treated switch, whether alone or in combination with high SA levels, notably at NaCl 125 mM + SA 0.75 mM. As a result of the current research, it can be determined that decreased Na+ levels in the shoots safeguard leaf growth and confer salt tolerance happening tissues, as evidenced by the condensed formation of necrotic shoot spots in plants treated with SA (0.5 mM).

Activities of antioxidant enzymes: Figure 5A-C shows the movement of CAT, SOD, and APX in response to SA and NaCl supplementation. In 125 mM NaCl-treated plants, SOD activity increased by 5.27 times, CAT activity increased by 3.28 times, and APX activity increased by 7.63 times when compared to control. In comparison to NaCl alone plants, SA supplementation increased SOD action via 1.31 times with 0.50 mM SA and 1.76 fold with 0.75 mM SA. When contrasted to NaCl only treated plants, 0.50 mM NaCl increased CAT and APX activities by 1.77 and 2.12 times, correspondingly. In comparison to NaCl-only treated plants, SA (0.75 mM) boosted CAT activity via 2.51 fold as well as APX activity via 2.22 fold (Fig. 5C-D).

Several plant species, including *Azolla*, *Medicago truncatula* (Mhadhbi, 2011; Masood *et al*., 2012), broad bean, as well as soybean, have been observed to benefit from increased antioxidant activity (Mohamed & Shaddad, 2013). Higher ROS scavenging through increased antioxidant enzyme activity enhanced salt tolerance in soybean, according to Alscher *et al*., (2002). In the present work, SA increased salt stress tolerance by increasing the activities of antioxidant enzymes. These findings support Ahmad *et al*., (2011) findings in *Brassica juncea* and findings in *Oryza sativa* (Guo *et al*., 2007). Deficiency stress in zoysiagrass, scary stress in maize (Chen *et al*., 2014; Janda, 1999), and stress of heavy metal in rice have all been documented to promote antioxidant enzyme activity (Mishra & Choudhuri, 1999). SA-induced increases in CAT and APX activity in soybean plants helped to quickly neutralise H_2O_2 and hence shield membranes from the harmful effects of stress.

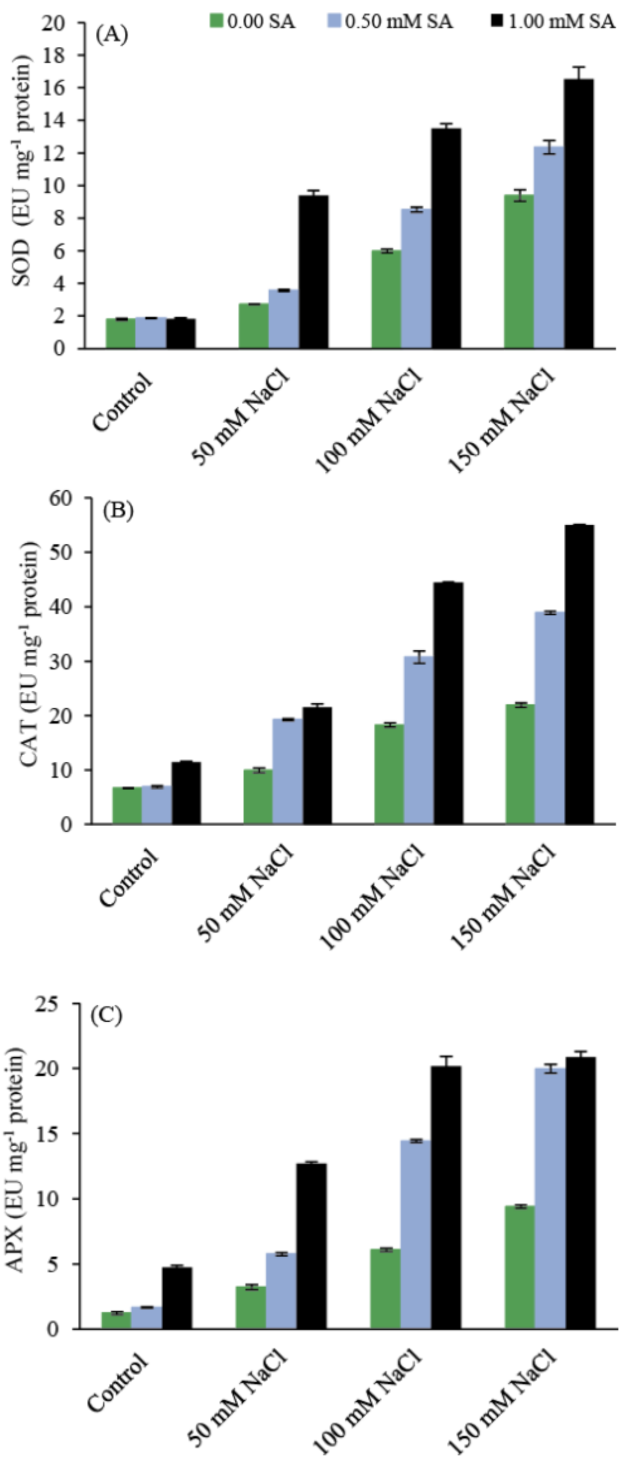


Fig. 5: The influence of salicylic acid in addition to various NaCl concentrations on the activity of SOD, CAT, and APX in soybean plants. The values represent the M & SD of three separate replications (n = 5).

Conclusion

This study shows that NaCl stress has a detrimental influence on soybean seedling development and physio-biochemical characteristics. SA supplement, on the other hand, boosted development and growth by modulating osmoprotectants and increasing the activity of the enzymatic antioxidant scheme (SOD, APX, and CAT). SA significantly decreased H_2O_2 and MDA buildup, as well as membrane permeability. SA also decreases the absorption of Na+ ions in the shoot and root while increasing K+ uptake. As a result, it is clear that applying an optimum SA concentration to soybean seedlings may be regarded an effective stress alleviation approach.

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