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Influence of salicylic acid on biochemical parameters and antioxidant system in mashbean plants grown under salt stress conditions

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Abstract: Abiotic stress factors affect almost every aspect of physiology and biochemisrtry of a plant. The present study investigates the role of salicylic acid (SA) in inducing plant tolerance to salinity. The application of 0.5 mM and 1.0 mM SA to mashbean (*Vigna mungo* L.) plants provided protection against 30mM or 45mM NaCl stress through elevated antioxidant system. The genotypes KUG 363, KUG 310, (salt sensitive), KUG 502 and KUG 529 (salt tolerant) along with UL 338 (as check) were subjected to salt stress. Relative leaf water content (61%) decreased under 45mM salt stress in salt tolerant genotype KUG 529 as compared to control (85%). Leaf water potential was also recorded at 50 DAS in salt tolerant genotype KUG 529 (-2.66 mpa) and in salt sensitive genotype KUG 363(-3.76 mpa) .All the genotypes showed higher accumulation of Reactive Oxygen Species under salt stress. A remarkable decrease was shown in antioxidant enzymes like catalase (179 micro mole/min/g FW) and ascorbate peroxidase (1617 n moles/min/g FW) in KUG 529 . The level of antioxidant system was enhanced catalase (184 micro mole/ min/g FW) and ascorbate peroxidase (1853 n moles/min/g FW) in mashbean plants under NaCl stress following SA applications . Thus SA helped in conferring stress tolerance to mashbean plants through enhanced antioxidant system. However, tolerant genotypes responded better than sensitive ones and lower concentration of SA (0.5mM) was more effective.

Keywords: Antioxidant enzymes, Mashbean, Salicylic acid, Salt stress

INTRODUCTION

The major problems in achieving higher yield are lack of exploitable genetic variability, poor harvest index and susceptibility to biotic and abiotic stress. Abiotic stress such as drought, salinity, extreme temperatures and chemical toxicity are serious threats to agriculture. Salt stress adversely affects crop productivity and quality. Salt stress is one of the major abiotic stress factors that affect almost every aspect of the physiology and biochemistry of the plant, resulting in reduction in its yield (Fahramand *et al.,* 2014). Punjab is severely affected by salinity problem. About 85,000 ha of agricultural land is affected by waterlogging and salinity (Anonymous, 2009). Further, ground water in many parts of Punjab contains high concentration of dissolved salts with electrical conductivity between 2 to 7 dS/m (Shakya and Singh, 2010). Salinity has plagued soil fertility and drastically affected growth and survival of crop plants. A steady decrease in plant height under salinity stress was reported in cowpea (Hussein *et al.,* 2007). Salinity reduces plant growth, alters ionic relation by ionic and osmotic effects and induces oxidative stress (Parida and Das, 2005; Silva *et al.,* 2008; Molassiotis *et al.,* 2006). Decrease in number of seeds per pod, number of pods per plant and severe reduction in seed yield per plant under increased salinity levels was reported in mungbean genotypes (Ahmad *et al.,* 2005). Ghai *et al.* (2010) also recorded a significant decrease in yield percentage of mashbean genotypes grown under salt stress.

Mashbean (*Vigna mungo* L. Hepper) is the most important pulse crop in India and occupies a unique position in Indian agriculture. Its cultivation is about 3.26 million hectare and 1.74 million tones is its annual production with productivity of 5.34 Kg (Anonymous, 2012). Although India is the main producer of mashbean but its production is limited due to various biotic and abiotic stresses (Ghai *et al*.,2014). A decrease in root and shoot fresh and dry weights of strawberry plants growing under saline conditions was reported by Karlidag *et al.* (2009). Salinity also leads to oxidative stress in plants due to production of reactive oxygen species (ROS). ROS are generated during metabolic processes damage cellular functions and consequently leads to disease, senescence and cell death (Joseph and Jini, 2011). The antioxidant enzymes such as Catalase , Ascrbate peroxidase and peroxidase showed variations in their activites under salt stress. Plants respond to stress by the synthesis of signaling molecules. Signaling molecules have been identified in plants such as calcium, jasmonic acid, ethyl-

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ene and salicylic acid (SA). These activate a range of signal transduction pathways. Salicylic acid(SA) is as an important signaling molecule for modulating plant responses to environmental stresses (Breusegem *et al,* 2001). However, there are few reports on the effects of salicylic acid on changes in antioxidant enzymes, ROS, membrane permeability of mashbean under salt stress. The increased activity of antioxidant enzymes also helps in overcoming the adverse effects of salt induced oxidative damage (Andre *et al.,* 2006). Therefore, the present investigation was designed to assess the ameliorative effects of salicylic acid on salt stress in resistant and sensitive genotypes of mashbean.

MATERIALS AND METHODS

Seeds of five mashbean genotypes differing in salinity tolerance i.e. KUG 363 and KUG 310 (salt sensitive); KUG 502 and KUG 529 (salt tolerant) and UL 338 (recommended variety) were procured from the Department of Plant Breeding and Genetics, PAU, Ludhiana. NaCl (30 and 45 mM) was applied in split dose (at the time of sowing and 15 days after sowing). Controls were irrigated with tap water. Foliar application of SA @ 0.5 mM and 1mM was done at 25 DAS on salt stressed plants. All the genotypes were subjected to following treatments:

T_1 (Control), T_2 (NaCl 30mM), T_3 (NaCl 45mM), T_4 (NaCl 30+ SA 0.5mM), T_5 (NaCl 45+ SA 0.5mM), T_6 (NaCl 30+ SA 1.0mM), T_7 (NaCl 45+ SA 0.5mM).

The data on following physiological, biochemical and yield contributing parameters were studied at flowering stage (50DAS).

Relative water content (RWC): The relative water content was estimated based on Barz and Weatherley method (1962) and the relative water content (RWC) of leafs was calculated as: RWC = 100 × [(fresh mass - dry mass) / (saturated mass - dry mass)]. Saturated mass was determined after incubation of the leaf in water for 24 h at room temperature. Dry mass was measured following oven drying at 75 °C to a constant mass.

Leaf water potential: Leaf water potential was measured with Wescor water potential system.

Electrolyte leakage: Electrolyte leakage from membranes of leaf tissue was estimated by the method described by Bajji *et al*., 2001 and expressed as per cent electrolyte leakage.

Measurement of malonaldehyde content (MDA), hydrogen peroxide content and acitivites of antioxidant enzymes: All samples were prepared for MDA, H_2O_2 and enzyme analyses by homogenisation of the fresh tissue in a solution (4 ml g-1 fresh weight) containing phosphate buffer. After the homogenate was centrifuged at 12,000×g for 20 min at 4°C, the supernatant was used to determine the enzymatic activities (Cho and Park, 2000). The MDA content was measured using the thiobarbituric acid (TBA) reaction as described by Heath and Packer, (1969). Hydrogen peroxide content was estimated following the method given by Velikova *et al*., 2000.

The POD activity was determined according to the method of Chance and Maehly (1995). Changes in absorbance of the reaction solution at 470 nm were recorded every 20 seconds. The CAT activity was measured using the method of Dhindsa and Motowe, (1981). The ascorbate peroxidase (APX) activity was measured by the decrease in absorbance at 290 nm as the ascorbate was oxidised (Nakano and Asada, 1981).

The data was statistically analysed using CPCS1 software to calculate CD at 5% level of significance.

RESULTS AND DISCUSSION

Results of the present study showed that overall reduction in physiological and biochemical parameters are due to salt stress. Various genotypes differed significantly from each other in amelioration of NaCl stress by SA.

Relative leaf water content: Salt stress caused reduction in RLWC of the leaves at flowering stage of all the genotypes. Also this decrease (20%) was more pronounced in sensitive genotype (KUG 363) than the tolerant one (KUG 529) as shown in fig. 1. However treating the plants with SA caused an improvement in RLWC, with lower concentration of SA (0.5mM) being more effective in 30mM NaCl stressed plants and tolerant genotypes responded more than sensitive ones. A decrease in water availability under soil salinity causes osmotic stress, which leads to a decreased turgor. Reduced RLWC under the influence of salt stress has been reported by El-Bassiouny and Bekheta, (2005) in wheat and Mohammadreza (2012) in *Brassica napus*. SA treatments induced an increase in RLWC of the stressed plants compared to the non-treated plants in all these crops.

Leaf water potential: Increasing salinity caused a decrease in water potential i.e. it became more negative in all genotypes. Similar observations were recorded by Kaur (2009) in salt stressed mashbean plants.

Foliar application of SA (0.5mM and 1.0mM) caused an improvement in water potential to some extent as shown in fig. 2. 0.5mM. SA proved to be more effective in tolerant and sensitive genotypes at 30mM NaCl. A decrease in leaf water potential was reported by Parida & Das (2005) and Cheng-Wu Jin *et al*., 2012) in Kenaf cultivars when salinity stress was imposed. Salinity acts to inhibit plant access to soil water by increasing the osmotic strength of soil solution. As the soil dries, the soil solution becomes increasingly concentrated, thus lowering the soil water potential, thereby limiting the plant access to soil water. Kaur (2011) also reported an improvement in water potential of salt stressed mashbean plants following SA applications

Membrane stability index: Electrolyte leakage enables cell membrane injury to be assessed when plants are subjected to stress. The increase in membrane permeability was found to be in NaCl concentration de-

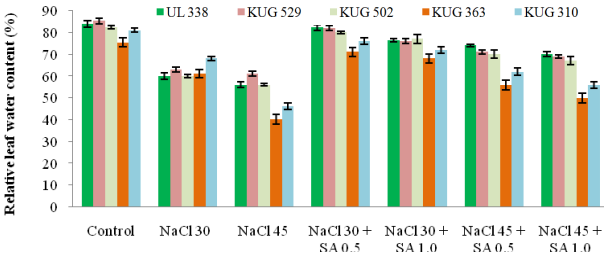

Fig. 1. *Effect of NaCl and SA treatments on relative leaf water content.*

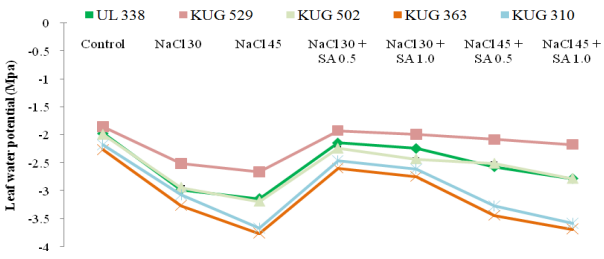

Fig. 2. *Effect of NaCl and SA treatments on Leaf water potential.*

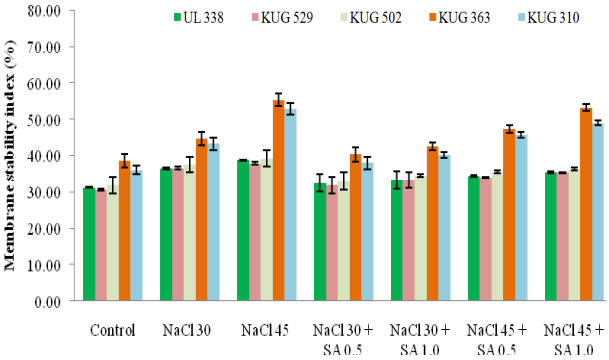

Fig. 3. *Effect of NaCl and SA treatments on membrane permeability.*

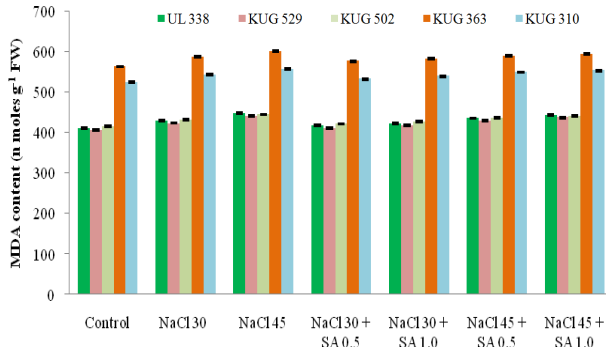

Fig. 4. *Effect of NaCl and SA treatments on MDA content.*

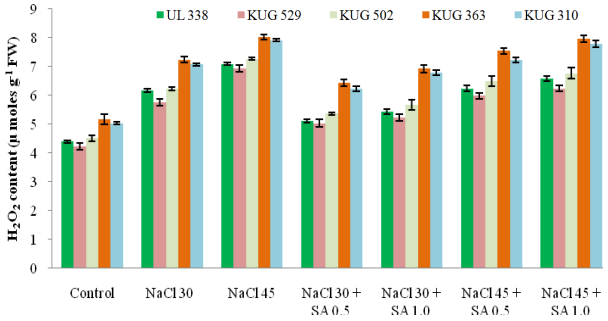

Fig. 5. *Effect of NaCl and SA treatments on hydrogen Peroxide content.*

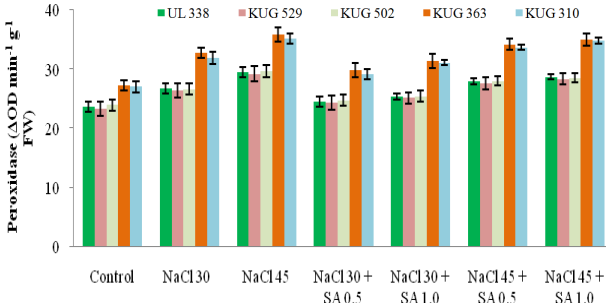

Fig. 6. *Effect of NaCl and SA treatments on peroxidase activity.*

pendent manner i.e. 45mM NaCl causing more electrolyte leakage from the membrane as compared to 30mM NaCl. Treatment of salt stressed plants with SA (0.5mM nd 1.0mM) helped in overcoming the adverse effects of salinity by restoring the membrane permeability. Salinity induced increase in electrolyte leakage and its amelioration by SA application has been reported earlier by Gunes *et al*. (2007) in maize and Khan *et al.,* (2010) in cucumber. Mohammadreza, (2012) also reported an increase in electrolyte leakage in salt stressed *Brassica napus* seedlings. SA facilitated maintenance of membrane functions through induction of antioxidant mechanisms.

Malondialdehyde content (MDA): Lipid peroxidation measured as MDA content is considered to be an indicator of oxidative damage from stress (Sevengor *et al.,* 2011). In the present investigation, salt sensitive genotypes showed more accumulation of MDA content as compared to salt tolerant ones and application of SA (0.5 and 1mM) helped in reducing its level. Simaei *et al.* (2011*)* also observed that MDA content declined in soybean treated with SA in the presence of 50 mM NaCl. Salinity significantly increased MDA content in Basil plants and treating the plants with SA decreased lipid peroxidation decreased based on MDA measurement in the salt stressed plants (Delavari *et al.,* 2010). Our study revealed that 0.5mM SA was more effective in reducing the MDA content in all the genotypes growing under 30mM NaCl.

Hydrogen peroxide content (H_2O_2): Under the influence of salt, H_2O_2 content increased in mashbean genotypes. However, application of SA declined the concentration of H_2O_2 in all the genotypes with more decline in tolerant as compared to sensitive genotypes.

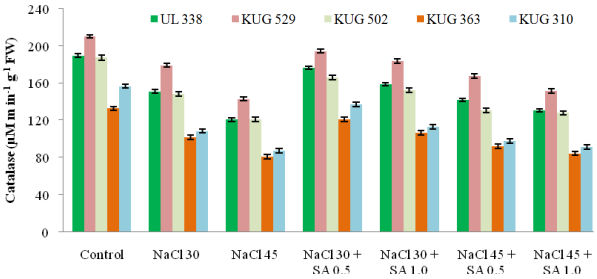

Fig. 7. *Effect of NaCl and SA treatments on catalase activity.*

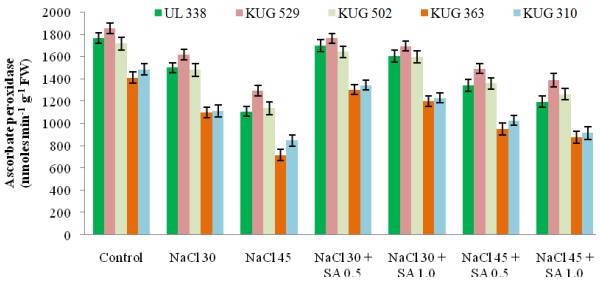

Fig. 8. *Effect of NaCl and SA treatments on ascorbate peroxidase activity.*

Table 1. Effect of NaCl and salicylic acid treatments on number of pods plant^{-1} in mashbean genotypes.

Treatments	Genotypes				
	UL 338	KUG 529	KUG 502	KUG 363	KUG 310
Control	39.2±1.85	44.5±0.87	31.4±1.33	27.8±0/74	29.2±0.74
NaCl 30	30.5±1.73	30.3±0.95	24.5±1.15	17.6±0.70	18.4±1.48
NaCl 45	29.8±1.67	29.5±1.28	21.2±1.73	15.2±0.84	16.5±1.42
NaCl 30 + SA 0.5	37.0±0.69	38.2±1.44	30.6±0.16	24.7±0.87	26.5±1.15
NaCl 30 + SA 1.0	36.5±0.67	36.1±1.45	30.4±1.85	22.5±0.91	25.7±0.92
NaCl 45 + SA 0.5	32.6±0.67	34±1.27	29.7±0.87	20.8±1.73	21.3±0.85
NaCl 45 +SA 1.0	30.0±0.70	32.6±1.50	28.1±0.91	19.6±1.96	20.6±0.89
CD (p=0.05) Genotypes = 1.31, Treatments = 1.55, Genotypes x Treatments = NS					

Table 2. Effect of NaCl and salicylic acid treatments on seeds pod^{-1} in mashbean genotypes.

Treatments	Genotypes				
	UL 338	KUG 529	KUG 502	KUG 363	KUG 310
Control	6.1±0.29	6.3±0.18	6.0±0.24	5.3±0.08	5.4±0.12
NaCl 30	5.3±0.12	5.4±0.12	5.6±0.14	4.7±0.10	4.8±0.20
NaCl 45	4.9±0.13	5.2±0.13	5.3±0.14	4.2±0.08	4.4±0.21
NaCl 30 + SA 0.5	6.0±0.10	6.0±0.12	5.7±0.13	4.9±0.12	5.0±0.19
NaCl 30 + SA 1.0	5.8±0.17	6.0±0.24	5.8±0.16	5.1±0.09	5.1±0.23
NaCl 45 + SA 0.5	5.4±0.21	5.5±0.23	5.9±0.08	4.8±0.19	5.0±0.10
NaCl 45 + SA 1.0	5.5±0.19	6.0±0.25	5.8±0.08	4.9±0.18	4.9±0.10
CD (p=0.05) Genotypes = 0.18, Treatments = 0.21, Genotypes x Treatments = NS					

Table 3. Effect of NaCl and salicylic acid treatments on 100-seed weight (g) of mashbean genotypes.

Treatments	Genotypes				
	UL 338	KUG 529	KUG 502	KUG 363	KUG 310
Control	4.28±0.17	4.44±0.15	4.29±0.17	4.04±0.13	4.05±0.28
NaCl 30	3.51±0.18	3.95±0.09	3.88±0.23	3.45±0.10	3.57±0.29
NaCl 45	3.42±0.16	3.83±0.12	3.68±0.19	3.26±0.20	3.50±0.21
NaCl 30 + SA 0.5	4.07±0.22	4.40±0.11	4.15±0.17	3.98±0.21	4.01±0.31
NaCl 30 + SA 1.0	4.03±0.14	4.12±0.20	4.04±0.31	3.86±0.25	4.00±0.17
NaCl 45 + SA 0.5	3.98±0.12	4.01±0.06	4.00±o.29	3.76±0.24	3.78±0.25
NaCl 45 + SA 1.0	3.91±0.14	3.99±0.10	3.98±0.18	3.66±0.23	3.77±0.30
CD (p=0.05) Genotypes = 0.21, Treatments = 0.21, Genotypes x Treatments = NS					

0.5mM SA caused 13% reduction in tolerant genotypes while sensitive genotypes showed 11% reduction in H_2O_2 content in plants growing under 30mM NaCl (Figure 5). The serious damage caused by salt stress is at least partially due to the generation of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide. An increase in H_2O_2 content in the wheat seedlings under high salinity was observed by Erdal *et al.* (2011). Of all the genotypes studied KUG 529 showed significantly lesser H_2O_2 content which might be contributing towards its salt tolerant behavior.

Peroxidase activity (POX): Application of 45mM NaCl caused more increase in POX activity in leaves of mashbean genotypes as compared to 30 mM NaCl

Table 4. Effect of NaCl and salicylic acid treatments on seed yield plant^{-1} (g)of mashbean genotypes.

Treatments	Genotypes				
	UL 338	**KUG 529**	**KUG 502**	**KUG 363**	**KUG 310**
Control	10.36±1.39	12.81±0.17	8.16±1.00	5.89±0.26	6.26±0.75
NaCl 30	5.69±0.50	6.46±0.21	5.37±0.69	2.87±0.26	3.11±0.13
NaCl 45	4.99±0.38	5.86±0.23	4.10±0.02	2.18±0.20	2.64±0.19
NaCl 30 + SA 0.5	9.01±0.16	10.07±0.32	7.25±0.42	4.81±0.31	5.26±0.04
NaCl 30 + SA 1.0	8.45±0.19	8.90±0.42	7.13±0.79	4.44±0.39	5.22±0.26
NaCl 45 + SA 0.5	6.99±0.09	7.48±0.13	6.97±0.21	3.84±0.71	4.01±0.19
NaCl 45 + SA 1.0	6.43±0.23	7.77±0.16	6.46±0.01	3.60±0.70	3.78±0.22
CD (p=0.05) Genotypes = 0.49, Treatments = 0.58, Genotypes x Treatments = NS					

Concentrations used were in mM.

stress. Treating the plants with SA (0.5mM and 1.0mM) decreased the activity level of POX in all the genotypes including check variety UL 338.

Erdal *et al.* (2011) reported that salt stress significantly increased the activity of POX in wheat genotypes. Foliar applications of SA caused a reduction in activity level of POX as compared to salt grown plants, but it was more than non- saline control plants. Purcarea and Cachiţa-Cosma, (2010) observed a decrease in activity of POX enzyme under the combined influence of salt and SA in maize.

Catalase activity (CAT): Figure 7 shows the effect of SA on the catalase activity of mash genotypes grown under salt stress. Salt stress at both the concentrations decreased the catalase activity and this decrease was in a concentration dependent manner. However, under the influence of SA, an increase in activity was recorded and it was more pronounced in the salt-tolerant than in the salt-sensitive genotypes. Genotype KUG 529 showed maximum increase in catalase activity with application of 0.5mM SA. NaCl induced inhibition of CAT activity was also recorded in wheat (Erdal *et al.,* 2011). According to Simaei *et al.* (2011) application of SA increases the activity of antioxidant enzymes and could ameliorate the toxic effects generated from NaCl in soybean.

Ascorbate peroxidase activity(APX): All the genotypes showed a reduction in the activity level of APX, when subjected to salt stress as depicted in Figure 8. 45mM NaCl caused more decline in APX activity as compared to 30mM NaCl stress. Application of SA (0.5mM and 1.0mM) increased the activity level of APX in all the genotypes including check variety UL 338. Lower concentration of SA when applied to 30mM NaCl stressed plants caused a significant increase in APX activity of all the genotypes. Sevengor *et al.* (2011) reported that on exposure to 100 mM NaCl, exogenous SA application significantly increased the activity of APX in pumpkin genotypes

Yield and yield contributing parameters: Salt stress significantly decreased yield and various yield contributing parameters in all the genotypes. It is envisaged from table 1,2,3&4 that foliar application of SA helped in overcoming the deleterious effects of salinity by improving the yield attributes in plants grown under salt stress. 45mM salinity level caused about 14% decrease in number of seeds in tolerant varieties and about 20% in sensitive ones. Similarly, the number of pods per plant decreased by about 32% in tolerant genotypes and about 45% in comparison to their respective controls. 100-seed weight and seed yield per plant also showed a similar trend.

Salinity induced reduction in yield has also been reported by Ghai *et al.* (2010) in mashbean. Improvement in yield following SA application under salt stress has been worked out in mungbean by Pooja and Sharma (2010).

Conclusion

Thus, salt stress limits plant growth by adversely affecting various physiological and biochemical processes. As SA was effective in inducing stress tolerance when applied as a foliar spray it appears that SA has regulatory role in activating biochemical pathways associated with salt tolerance mechanisms. The ameliorative effect of SA might be linked to the observable increase in RLWC and water potential of leaves. SA treatments also enhanced the level of antioxidant system (CAT and APX in mashbean plants under NaCl stress and reduced the hydrogen peroxide and malondialdehyde content. The elevated antioxidant system counters the oxidative stress as well as other direct effects of NaCl stress in plants that helped in overcoming the deleterious effects of salinity and improved the yield contributing parameters. Also lower concentration of SA proved to be more effective to ameliorate the adverse effects of salt stress.

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