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Research Article

IMPROVING CHICKPEA PERFORMANCE BY EXOGENOUSLY APPLIED SALICYLIC ACID UNDER SALT STRESS- CHANGES IN BIOCHEMICAL ASPECTS AND ANTIOXIDANT ENZYMES

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ABSTRACT
After poaceae, legumes belong to second most popular family and are grown for their nutritive protein rich seeds. Chickpea has special importance among the grains especially in arid and semi-arid regions and is sensitive to salinity. Therefore, it becomes necessary to make a plan to mitigate the salinity effect on this plant. For this purpose, an experiment was conducted in net house of Department of Botany, Kurukshetra University, Kurukshetra to investigate the role of salicylic acid (SA) at different concentrations (10^{-4}, 10^{-5} and 10^{-6} M) in overcoming salinity stress imposed on chickpea plants in natural conditions. SA treatment was given in two forms: pre-soaking and foliar spray. Different salinity levels (0, 50 mM, 100 mM and 150 mM) were applied and caused significant reduction in photosynthetic pigments and other biochemical parameters. Our main findings are as follows: (1) Salt stress has detrimental effect on growth and physiology of plants whereby affecting protein, chlorophyll content, lipid peroxidation and causing oxidative stress. (2) Application of SA at 10^{-5} M was the most significant concentration in modulating the inhibitory effects of salt stress. (3) Foliar spray treatment was 10-15 % more effective than pre-soaking seed treatment in alleviating salt stress.

Keywords: *Chickpea, Salicylic Acid, Salt Stress, Lipid Peroxidation, Antioxidative Enzymes*

INTRODUCTION
Salt stress is one of the major abiotic stresses that adversely affect the global crop production and its adverse impacts are getting more serious in the regions where saline water is used for irrigation. It induces reactive oxygen species, alters the activity of antioxidant system and adversely affects the process of photosynthesis (Flowers, 2004; Koca *et al*., 2007). Various strategies have been employed to mitigate the deleterious effect of salt stress. Presently, the recommended strategies to overcome the adverse effects of salt stress include the use of ameliorative water management, tolerant cultivars and diverse cultural practices. Abiotic stresses like light, heat, cold, salinity, UV rays and also heavy metals are responsible for alteration in osmotic and ionic homeostasis, ultimately, damage to structural and functional proteins of plant cells (Bohra and Sanadhya, 2015; Sanadhya *et al*., 2013). Abiotic stresses are the most vital restraining factor in crop establishment. Extent and nature of both stresses differs with the developmental stages of plants particularly during germination, reproductive and maturation stage (Chauhan *et al.,* 2015) that leads to fall in yield. The germination stage is affected first of all because at this stage plants are more sensitive to abiotic stresses than other growth and developmental stages (Luan *et al.,* 2014). Salinity stress limit the seed germination and seed establishment of plants growing in arid and semi-arid areas (James *et al.*, 2002).

In the past few decades, among many strategies used to combat the deleterious effects of salinity stress, exogenous application of plant growth regulators has received considerable attention. Salicylic acid (SA) is known as an endogenous growth regulator and causes biotic and abiotic stress tolerance in crops (Joseph *et al*., 2010; Javid *et al.,* 2011). The role of SA is important in seed germination, fruit yield (Hayat *et al.,* 2010), photosynthetic rate (Khan *et al*., 2003), enzymatic activity (Dolatabadian *et al.,* 2008), plant growth and yield (Hussein *et al.,* 2007) and uptake and transport of ions (Afzal *et al.,* 2005) have been well addressed. Rafique *et al.,* (2011) reported the response of pumpkin (*Cucurbita pepo* L.) to exogenously applied SA regarding salt and drought tolerance. Earlier studies on seed germination of

chickpea from our laboratory also exhibited positive effect of pretreatment of chickpea seeds with SA under salt stress (Savita and Jakhar, 2015).

Considering the above mentioned literature about the effects of SA it is proved that if SA is applied exogenously, it might enhance the salinity tolerance ability of chickpea. Hence, the study was carried out to find out the effects of SA on biochemical and antioxidant enzymes activities of chickpea under salt stress. The results of the present study can be helpful to create tolerance capacity of chickpea under abiotic stress conditions and also elucidate the role of exogenously applied SA in salinity.

MATERIALS AND METHODS

Foliar Spray Treatment

The certified seeds of chickpea (*Cicer arietinum* L.) were purchased from CCS Haryana Agriculture University, Hisar. The seeds were surface sterilized with 0.01% mercuric chloride solution followed by inoculation with Rhizobium and were sown in earthen pots (0.254 m in diameter) filled with sandy loam soil and farmyard manure (6:1) arranged under a simple randomized block design in the net house of the Botany Department of Kurukshetra University, Kurukshetra during the winter season (November–February). Fifteen days old plants were maintained under different saline concentrations of 0, 50, 100 and 150 mM. At 30 days after sowing (DAS), the foliage of the plants were sprayed uniformly with double distilled water (control), with 10^{-4}, 10^{-5} and 10^{-6} mol/L concentration of SA dissolved in ethanol to elucidate the effect of SA on plants. The plants were sampled at 45 DAS to assess various biochemical parameters and antioxidant enzymes activities.

Presoaking Seed Treatment

The certified seeds of chickpea (*Cicer arietinum* L.) were purchased from CCS Haryana Agriculture University, Hisar. The seeds were surface sterilized with 0.01% mercuric chloride solution followed by inoculation with Rhizobium and different concentrations of salicylic acid (10^{-4}, 10^{-5} and 10^{-6} mol/L). These presoaked seeds were sown in earthen pots (0.254 m in diameter) filled with sandy loam soil and farmyard manure (6:1) arranged under a simple randomized block design in the net house of the Botany Department of Kurukshetra University, Kurukshetra during the winter season (November–February). Fifteen days old plants were maintained under different saline concentrations of 0, 50, 100 and 150 mM. The plants were sampled at 45 DAS to assess various biochemical parameters and antioxidant enzymes activities.

Estimation of Chlorophylls and Carotenoids

Chlorophylls and carotenoids are estimated by following methods. Leaf sample (200 mg) was ground in chilled 80% acetone (AR grade) with 20 mg of $CaCO_3$ and centrifuged at 3000 g for 5 min. Absorbance of the filtrate was recorded at 645 and 663 nm for chlorophylls and at 480 and 510 nm for carotenoids depending upon respective peaks in their absorption spectra using a UV-Visible spectrophotometer. Chlorophyll (Chl) amount was estimated with the formula of Arnon (1949). Carotenoid level was calculated by the method of Holden (1965).

Estimation of Lipid Peroxidation

The level of lipid peroxidation in samples was measured by estimating the malondialdehyde (MDA) present (Heath and Packer, 1968). Leaf samples (0.2 g) were homogenized in 3 mL of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15000 g for 15 min. To 1.0 ml aliquot of the supernatant, 2.0 ml of 0.5 % thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The mixture was heated at 95°C for 30 min in a water bath and then cooled in an ice bath. After centrifugation at 10000 g (Remi) for 10 min the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption of each sample at 600 nm was recorded and subtracted from the absorbance recorded at 532 nm.

Estimation of Total Soluble Protein

Total soluble proteins were estimated according to the method described by Bradford (1976) using Coomassie Brilliant Blue G-250. Fifty mg of fresh leaf tissue (earlier stored in a freezer) was dropped boiling 80% ethanol (EtOH) on a water bath for a minute. The tissue along with EtOH was cooled to

room temperature and homogenized. The extract was centrifuged at 10,000 g for 5 min. The residue was re-extracted with 5% perchloric acid followed by centrifugation at 10,000 g for 5 min. Five-mL of 1N NaOH was added to the residue and maintained in warm water (40-50°C) with regular shaking for 30 min. The clear supernatant was used for further analysis.

Estimation of Proline

Proline was determined according to the method described by Bates *et al.,* (1973). Approximately, 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 2 filter paper.

Two ml of the filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm. Appropriate proline standards were included for the calculation of proline in the samples.

Measurement of peroxidase (POD) Activity

Total peroxidase activity was measured by the method of Maehly and Chance (1954). Plant material (0.1 g) was homogenized with ice cold distilled water and centrifuged in a Remi centrifuge at 6000 g for 10 min. The supernatant was used as the enzyme source and final volume of the extract raised to 10 mL with ice cold double distilled water.

The reaction set was prepared by mixing 2 mL each of enzyme source; phosphate buffer (pH 7.0); guaiacol (20 mM), and H_2O_2 (10 mM) in sequence. A blank set was prepared by mixing 2 mL of enzyme source; 2 mL of phosphate buffer (pH 7.0) and 4 mL of double distilled water. Blank, and reaction sets, were kept undisturbed at room temperature exactly for 10 min., then, the absorbance was recorded in a spectrophotometer at 420 nm. Protein was estimated from the same extract following the procedure of Bradford (1976).

Measurement of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was measured by the method of Giannopolitis and Ries (1977). Fifty-mg of fresh leaf tissue was crushed in 2 mL of 0.1M EDTA- phosphate buffer, pH 7.8, containing K_2HPO_4 and EDTA and the final volume raised to 100 mL with double distilled water (DDW). This was centrifuged at 15000 g and the resultant supernatant used as crude extract. The reaction mixture was prepared by adding 0.1 mL of crude extract followed by 0.9 mL of DDW, 0.5 mL of 300 mM Na_2CO_3 (pH 10.2), 0.5 mL of 378 µM p-nitrobluetetrazolium chloride (NBT), 0.5 mL of 78 mM L-methionine and 0.5 mL of 7.8 µM riboflavin.

The final reaction mixture was 3 mL. The reaction was carried out in test tubes at 25°C for 15 min under 100 µmol photon $m^{-2}s^{-1}$ PFD from fluorescent lamps. The initial rate of reaction, measured by the difference in increase in absorbance at 560 nm in the presence, and absence, of extract was proportional to the amount of enzyme. The unit of SOD activity was obtained as that amount of enzyme which under the experimental conditions.

Measurement of Catalase (CAT) Activity

The catalase activity was measured by following the method of Aebi (1984). The reaction mixture was prepared by adding 1.5ml of 50mM HEPES buffer, 1.2 ml of 150mM H_2O_2 and 30 µl petal extract. In the mixture without enzyme, no crude extract was added, instead of it 50 µl 50 mM HEPES buffer was added.

The change in absorbance was read at 490 nm in the test tube cuvette using uv-vis spectrophotometer. Specific activity of catalase was expressed in terms of per mg protein. Protein was estimated from the same extract following the procedure of Bradford (1976) as described earlier.

Statistical Analysis

A mean of three readings was taken in every replication. In biochemical estimation, three aliquots were used for each replication. Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) version 16.0. Two-way ANOVA was used to test whether there was a significant difference in various estimations.

RESULTS AND DISCUSSION

Photosynthetic Pigments

Salinity stress significantly declined the photosynthetic pigments (chl.a, chl.b, total chl. and carotenoids) on 45th DAS (Tables 1-2). Compared with the control, highest level of salinity reduced chl.a by about 62%, chl.b by about 50%, total chl. by about 59% and carotenoids by about 62%. However, spraying with SA (10^{-5}M) mitigated NaCl-induced effect by increasing chl.a by about 38%, chl.b by about 22%, total chl. (a+b) by about 36% and carotenoids by about 28% corresponding to their respective controls while seed presoaking treatment with 10^{-5}M SA increased chl.a by about 22%, chl.b by about 12%, total chl. (a+b) by about 19% and carotenoids by about 16% corresponding to their respective controls under 50mM salt stress. Moreover, it was also observed that 10^{-6}M SA has less significant effects on photosynthetic pigments in both treatments. The effectiveness of SA applied as foliar spray depends on the type of species, time of application and the concentration used (Hayat *et al.,* 2010). The investigation was done to improve our understanding of the effect of the various concentrations of SA applied as foliar spray and as seed priming on the photosynthetic pigments, biochemical and antioxidative enzymes in chickpea and to find out the most effective concentration of SA. Our results showed that salinity decreased the photosynthetic pigments which are in conformity of previous reports Baber *et al.,* (2014) who reported that salinity caused a marked reduction in photosynthetic pigments in fenugreek which might be due to the possible oxidation of chlorophyll and other chloroplast pigments coupled with instability of the pigment protein complex under salt stress. In our study, reduction in chlorophyll content was mitigated by the foliar application of SA and seed priming with SA. Similarly, Abreu and Munne (2009) also revealed that SA deficiency is associated with reduced damage to the photosynthetic apparatus as well as chlorophyll levels. Further in another study, Fahad and Bano (2012) also noticed that salt stress significantly decreased the total chlorophyll content of leaves of maize plant.

Lipid Peroxidation

Lipid peroxidation (MDA) increased in the presence of salt stress environment indicating that S_3 level of salinity is lethal to plants. The effect of foliar application as well as seed presoaking treatment with different concentrations of SA under salt stress on MDA content of chickpea plants are shown in table 3 and 4. In the present study, an increase of about 110% in MDA content was observed in 150mM level of salinity whereas about 34% increase was observed under 100mM level of salinity. Under stress and non-stress conditions, the effect of 10^{-5}M SA was more pronounced than 10^{-4}M and 10^{-6}M SA. Compared with the corresponding controls, foliar application and seed presoaking treatment with 10^{-5}M SA lowered MDA content by about 18% and 6%, 28% and 19% and 31% and 21% at 50 mM, 100 mM and 150 mM level of salinity respectively. Furthermore, SA at 10^{-6}M was the least effective in modulating MDA levels under salinity stress. Our experimental results indicated that the end product of lipid peroxidation i.e. MDA content accumulated under salinity stress and exogenous application of SA lowered the MDA content. Our findings are in agreement with previous reports Kukreja *et al.,* (2005) who noticed the significant enhancement in lipid peroxidation in *Cicer arietinum* roots under salinity stress. Similar increase in MDA content has also been noted in *Cicer arietinum* L.cv. Gocke (Eyidogan and Oz, 2007). Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction (i.e. lipid peroxidation). The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Small hydrocarbon fragments such as ketones, MDA are formed by lipid peroxidation (Weckx and Clijsters, 1996). Morever, SA reduces MDA after the priming of faba bean under saline conditions (Azooz, 2009).

Protein Content

Data illustrated in tables 3-4 shows that SA as both foliar spray and seed presoaking treatment increased total leaf protein compared to the untreated plants under all levels of salinity stress. Highest level of salinity drastically reduced protein content (78%) over non-stressed plants whereas foliar application of 10^{-5}M SA significantly increased total protein content by about 48% and 29% at 100 mM and 150mM salinity levels respectively over control. Seeds primed with 10^{-5}M SA enhanced protein content by 29% and 20% at 100 mM and 150mM salinity levels respectively over control. At lowest salinity level, 10^{-5}M

SA enhanced protein content by 42% (foliar) and 30% (seed-presoaking) over control. Seeds primed with SA significantly affected the amount of protein under stressed as well as non-stressed conditions. Sarkar *et al.,* (2013) reported the occurrence of degradation and oxidation of proteins under salt stress. Furthermore, Song *et al.,* (2011) added that alleviation of degradation of proteins occurs by interactions between SA, NO and ABA under salt stress. Application of SA in soybean would increase protein content (Kumar *et al.,* 1996). Noctor and Foyer (1998) reported that free radicals produced under salt stress conditions may damage the proteins and reduces its content.

Proline

The effect of foliar application as well as seed presoaking treatment with different concentrations of SA under salt stress on proline content of chickpea plants are shown in tables 3-4. The accumulation of proline increased as salinity level increased. In the present study, an increase of about 191% in proline content was observed at 150 mM level of salinity whereas about 132% increase was observed under 100 mM level of salinity. Also, the foliar spray of SA at different concentrations increased the proline content under stress and non-stress conditions. The effect of 10^{-5}M SA was more pronounced than 10^{-4}M and 10^{-6}M SA. Foliar application with 10^{-5}M SA increased proline content by about 15%, 38% and 41% at 50 mM, 100 mM and 150 mM levels of salinity respectively. The same trait got enhanced by 8%, 36% and 30% by pre-soaking with 10^{-5}M SA at corresponding salinity levels. SA at 10^{-6}M was the least effective in modulating proline levels under salinity stress. In respect to proline content in salt stressed chickpea plants, application of salicylic acid at 10^{-5}M concentration showed better response significantly in relieving salt stress at salinity levels of 50 mM concentration when compared with only 50mM NaCl stressed plants. The mechanism of accumulation of compatible solutes also supported by the results obtained in this study. Proline, an amino acid act as a cytoplasmic osmoticum, a stabilizer for membrane and protein synthesis machinery, a hunter of free radicals, a sink for energy to regulate redox potential. Also, it serves to protect the protein against denaturation. The tolerance to osmotic and saline stress was associated with proline accumulation. Its concentration increases either by SA foliar spraying or salt stress. Wheat seedlings accumulated large amounts of proline under salt stress (Shakirova *et al.,* 2003) which provided enhanced tolerance against salinity stress (Yusuf *et al.,* 2008). Under salinity, plants require extra energy which could be provided by elevated sugar, protein and proline accumulation which are energy rich compounds (Banaras *et al.,* 2004). It can be proposed from this experiment that increased proline in plants treated with salicylic acid might be due to reduced breakdown of proteins and enhanced incorporation of individual amino acid into proteins. Proline supplements enhanced salt tolerance in olive (Oleaeuropaea) by amelioration of some antioxidative enzyme activities, the activity of photosynthesis, plant growth and the preservation of a suitable plant water status under salinity conditions (Gupta and Huang, 2014).

Antioxidant Enzymes Activity

The activities of antioxidant enzymes (viz. peroxidase, superoxide dismutase and catalase) were significantly enhanced by NaCl and/ or SA treatments (Figures 1-3) over control. All antioxidative enzymes were amplified linearly with SA addition and found utmost at highest salinity level (150 mM). The activities of SOD, POD and CAT were found to be higher (136, 203 and 415 % respectively) in response to 150mM NaCl concentration than that of 50mM salinity level (43, 52 and 147 % respectively). Foliar spray with 10^{-5}M SA was more productive than priming. The obtained results revealed that seed presoaking treatment with 10^{-5}M SA enhanced the activities of SOD, POD and CAT by about 20%, 37% and 17% whereas foliar spray with 10^{-5}M SA intensified the activities of SOD, POD and CAT by about 41%, 62% and 75% at lowest (50 mM) salinity level corresponding to their control. However, at highest (150mM) level of salinity, seed presoaking treatment with 10^{-5}M SA enhanced the activity of POD significantly by about 36% whereas the same concentration was not significant in enhancing the activities of SOD and CAT. However, foliar application with same concentration of SA strengthened the activities of SOD, POD and CAT significantly by about 25%, 55% and 41% corresponding to their controls. However, the efficacy of 10^{-4} M and 10^{-6}M was subordinate than 10^{-5}M SA. NaCl stress is the generation of oxidative stress that results from increased level of ROS in cells exposed to stress (Schutzendubel and

Polle, 2002). The increase in activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) following SA application could be the indicator of buildup of a protective mechanism to reduce oxidative damage induced by salt stress. Salinity induces oxidative stress by inhibiting the carbon dioxide assimilation, exposing chloroplasts to excessive excitation energy, which in turn promotes the generation of ROS from triplet chlorophyll (Gosset *et al.*, 1994). Fahad and Bano (2012) reported that the saline condition resulted in significantly higher SOD activity of leaves in maize plants. Salinity tolerance supported the activity of antioxidant enzymes, such as SOD and with the accumulation of non-enzymatic antioxidant compounds (Gupta and Huang, 2014). Senaratna *et al.*, (2000) have suggested a similar mechanism to be responsible for SA-induced multiple stress tolerance in bean and tomato plants. SA can play a critical role in modulating the cell redox balance, thereby protecting the plants against the oxidative damage (Yang *et al.*, 2004). Catalase seems to be a key enzyme in salicylic acid induced stress tolerance since it was shown to bind salicylic acid in vitro (Chen *et al.*, 1993). Peroxidase activity was increased by SA application in plants subjected to various abiotic stresses (Kang and Salveit, 2002; Popova *et al.*, 2003). SA enhanced the antioxidant enzymes activities (POD, SOD and CAT) when sprayed exogenously to the salinity stressed plants (Szepesi, 2008; Yusuf *et al.*, 2008) as SA application during seed priming is known to be associated with enhanced SOD, CAT and APX activities in maize under chilling stress (Farooq *et al.*, 2008).

From this study, it can be concluded that SA can alleviate salinity stress in chickpea plants by modulating antioxidant machinery during the initial growth (figure 4).

Table 1: Effect of SA Foliar Treatment on Photosynthetic Pigments Grown under Salinity Stress at 45 DAS

Treatments		**Photosynthetic Pigments**			
Salinity (mM)	**SA (mole/L)**	**Chl. a (mg g^{-1} DW)**	**Chl. b (mg g^{-1} DW)**	**Total Chl.(a+b) (mg g^{-1} DW)**	**Carotenoids (mg g^{-1} DW)**
0	0	2.19 ± 0.23	0.278 ± 0.10	2.47 ± 0.37	1.75 ± 0.20
	10^{-4}	2.41 ± 0.26	0.301 ± 0.72	2.71 ± 0.26	1.86 ± 0.20
	10^{-5}	2.93 ± 0.29	0.380 ± 0.57	3.31 ± 0.23	2.08 ± 0.28
	10^{-6}	2.57 ± 0.32	0.331 ± 0.46	2.90 ± 0.34	1.92 ± 0.18
50	0	1.56 ± 0.20	0.251 ± 0.60	1.81 ± 0.26	1.39 ± 0.26
	10^{-4}	1.85 ± 0.23	0.257 ± 0.37	2.11 ± 0.14	1.44 ± 0.00
	10^{-5}	2.15 ± 0.46	0.306 ± 0.16	2.46 ± 0.23	1.78 ± 0.15
	10^{-6}	1.96 ± 0.17	0.272 ± 0.20	2.23 ± 0.25	1.53 ± 0.18
100	0	1.25 ± 0.23	0.182 ± 0.81	1.43 ± 0.20	1.05 ± 0.23
	10^{-4}	1.47 ± 0.22	0.206 ± 0.30	1.68 ± 0.23	1.15 ± 0.24
	10^{-5}	1.73 ± 0.18	0.250 ± 0.57	1.98 ± 0.20	1.34 ± 0.14
	10^{-6}	1.65 ± 0.20	0.224 ± 0.29	1.87 ± 0.15	1.24 ± 0.11
150	0	0.85 ± 0.08	0.141 ± 0.49	0.99 ± 0.17	0.67 ± 0.11
	10^{-4}	0.95 ± 0.03	0.144 ± 0.30	1.09 ± 0.18	0.75 ± 0.20
	10^{-5}	1.35 ± 0.20	0.181 ± 0.16	1.53 ± 0.23	0.96 ± 0.17
	10^{-6}	1.13 ± 0.18	0.160 ± 0.03	1.29 ± 0.14	0.85 ± 0.14
F value					
Salinity		2786.46	860.308	3332.40	2700.53
Treatment		579.83	176.972	686.612	322.977
Salinity*Treatment		18.94	8.510	21.162	4.726

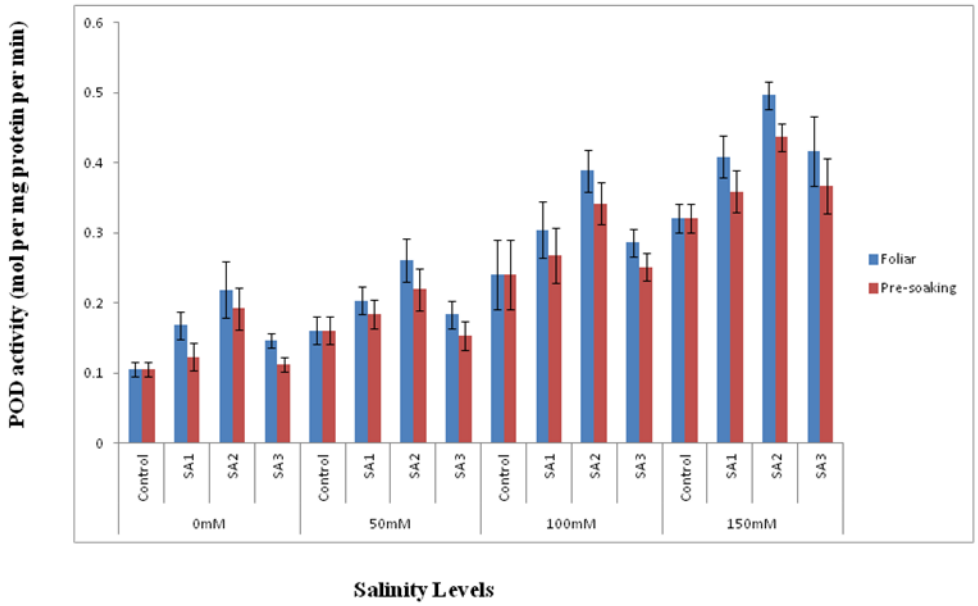

Figure 1: Effect of Foliar Spray and Pre-soaking Seed Treatment with Salicyclic Acid on POD Activity of Chickpea under Salt Stress

Table 2: Effect of SA Presoaking Treatment on Photosynthetic Pigments Grown under Salinity Stress at 45 DAS

Treatments		**Photosynthetic Pigments**			
Salinity (mM)	**SA (mole/L)**	**Chl. a (mg g^{-1} DW)**	**Chl. b (mg g^{-1} DW)**	**Total Chl.(a+b) (mg g^{-1} DW)**	**Carotenoids (mg g^{-1} DW)**
0	0	2.19 ± 0.36	0.278 ± 0.09	2.47 ± 0.26	1.75 ± 0.28
	10^{-4}	2.22 ± 0.25	0.292 ± 0.09	2.51 ± 0.23	1.84 ± 0.23
	10^{-5}	2.58 ± 0.22	0.334 ± 0.10	2.91 ± 0.32	1.93 ± 0.22
	10^{-6}	2.26 ± 0.36	0.305 ± 0.11	2.56 ± 0.29	1.89 ± 0.29
50	0	1.56 ± 0.20	0.251 ± 0.06	1.81 ± 0.23	1.39 ± 0.15
	10^{-4}	1.63 ± 0.23	0.259 ± 0.03	1.87 ± 0.46	1.45 ± 0.18
	10^{-5}	1.89 ± 0.19	0.281 ± 0.09	2.17 ± 0.17	1.61 ± 0.16
	10^{-6}	1.72 ± 0.15	0.277 ± 0.08	1.99 ± 0.20	1.47 ± 0.12
100	0	1.25 ± 0.17	0.182 ± 0.02	1.43 ± 0.22	1.05 ± 0.24
	10^{-4}	1.29 ± 0.18	0.198 ± 0.03	1.49 ± 0.18	1.09 ± 0.23
	10^{-5}	1.52 ± 0.14	0.220 ± 0.01	1.74 ± 0.15	1.18 ± 0.12
	10^{-6}	1.45 ± 0.23	0.208 ± 0.02	1.65 ± 0.12	1.19 ± 0.11
150	0	0.85 ± 0.11	0.141 ± 0.01	0.99 ± 0.12	0.67 ± 0.17
	10^{-4}	0.93 ± 0.17	0.142 ± 0.05	1.07 ± 0.15	0.69 ± 0.19
	10^{-5}	1.19 ± 0.20	0.160 ± 0.06	1.35 ± 0.16	0.85 ± 0.14
	10^{-6}	1.00 ± 0.14	0.147 ± 0.02	1.147 ± 0.19	0.75 ± 0.09
F value					
Salinity		2776.46	820.308	3432.40	2760.53
Treatment		572.83	166.972	656.612	329.977
Salinity*Treatment		18.84	8.610	22.162	4.716

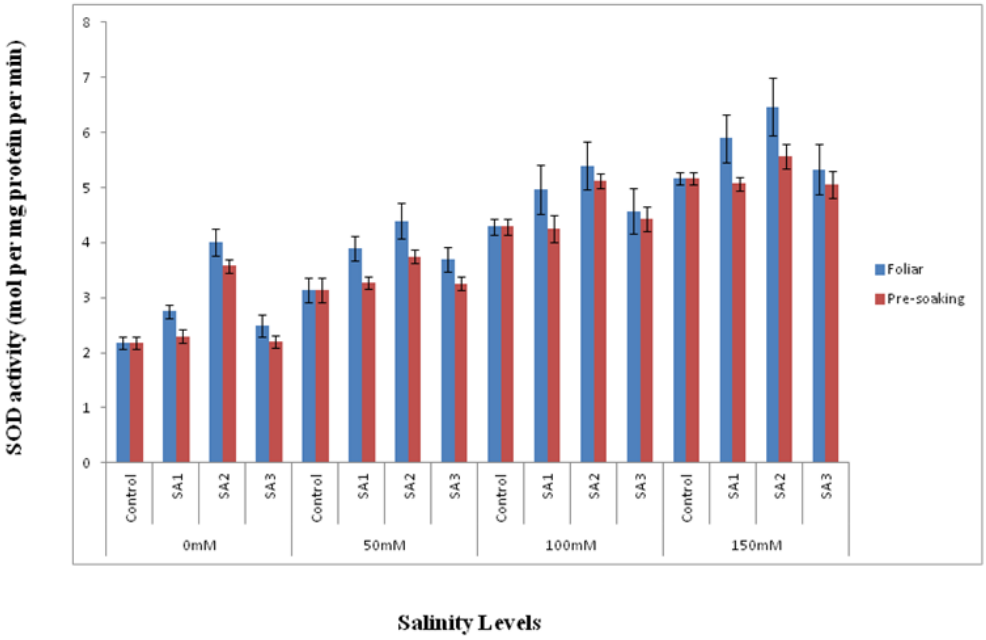

Figure 2: Effect of Foliar Spray and Pre-soaking Seed Treatment with Salicyclic Acid on SOD Activity of Chickpea under Salt Stress

Table 3: Effect of SA Foliar Treatment on Lipid Peroxidation, Protein and Proline Grown under Salinity Stress at 45 DAS

Treatments		**Biochemical Parameters**		
Salinity (mM)	**SA (mol/l)**	**Lipid Peroxidation (nmol g^{-1} DW)**	**Protein (mg g^{-1} DW)**	**Proline (µmol g^{-1}DW)**
0	0	0.701 ± 0.44	13.15 ± 0.14	122.36 ± 0.11
	10^{-4}	0.5919 ± 0.39	14.78 ± 0.14	191.17 ± 0.15
	10^{-5}	0.5635 ± 0.57	18.36 ± 0.21	216.88± 0.24
	10^{-6}	0.5963 ± 0.37	16.25 ± 0.15	157.84 ± 0.13
50	0	0.746 ± 0.31	9.79 ± 0.74	265.30 ± 0.18
	10^{-4}	0.6336 ± 0.37	10.63 ± 0.08	290.41 ±0.13
	10^{-5}	0.6178 ± 0.61	13.86 ± 0.55	306.14±0.13
	10^{-6}	0.6401 ± 0.00	11.71 ± 0.77	277.59±0.14
100	0	0.934 ± 0.19	5.89 ± 0.20	284.86±0.16
	10^{-4}	0.6978 ± 0.05	6.41 ± 0.05	353.22± 0.10
	10^{-5}	0.6694 ± 0.06	8.68 ± 0.10	393.90± 0.21
	10^{-6}	0.7515 ± 0.05	7.44 ± 0.66	343.11± 0.13
150	0	1.47 ± 0.06	2.90 ± 0.04	356.42± 0.15
	10^{-4}	1.0355 ± 0.01	3.43 ± 0.02	475.55± 0.17
	10^{-5}	1.0208 ± 0.01	3.75 ± 0.05	505.36± 0.29
	10^{-6}	1.1616 ± 0.02	3.72 ± 0.05	461.74± 0.12
F value				
Salinity		361.306	11920.23	20958.94
Treatment		21.945	760.23	1135.05
Salinity*Treatment		4.063	62.189	48.735

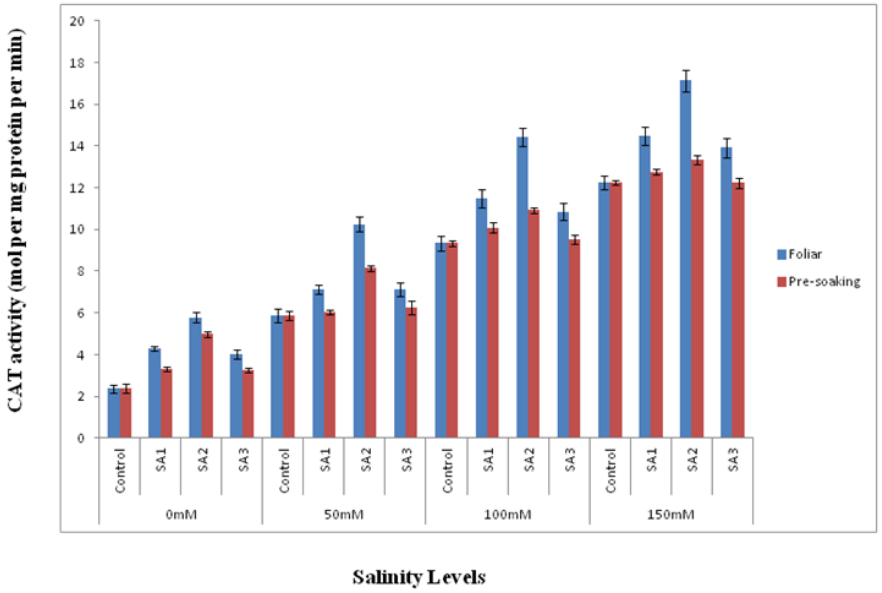

Figure 2: Effect of Foliar Spray and Pre-soaking Seed Treatment with Salicyclic Acid on CAT Activity of Chickpea under Salt Stress

Table 4: Effect of SA Presoaking Seed Treatment on Lipid Peroxidation, Protein and Proline Grown under Salinity Stress at 45 DAS

Treatments		**Biochemical Parameters**		
Salinity (mM)	**SA (mol/l)**	**Lipid Peroxidation (nmol g^{-1} DW)**	**Protein (mg g^{-1} DW)**	**Proline (µmol g^{-1}DW)**
0	0	0.701 ± 0.44	13.15 ± 0.14	122.36 ± 0.11
	10^{-4}	0.677 ± 0.39	13.01 ± 0.14	167.12 ± 0.15
	10^{-5}	0.640 ± 0.57	16.16 ± 0.21	191.17± 0.24
	10^{-6}	0.672 ± 0.37	14.31 ± 0.15	137.25 ± 0.13
50	0	0.746 ± 0.31	9.79 ± 0.74	265.30 ± 0.18
	10^{-4}	0.727 ± 0.37	9.96 ± 0.08	277.59 ± 0.13
	10^{-5}	0.702 ± 0.61	12.72 ± 0.55	286.56 ± 0.13
	10^{-6}	0.720 ± 0.00	10.31 ± 0.77	267.36 ± 0.14
100	0	0.934 ± 0.19	5.89 ± 0.20	284.86 ± 0.16
	10^{-4}	0..854 ± 0.05	5.95 ± 0.05	346.15 ± 0.10
	10^{-5}	0.760 ± 0.06	7.64 ± 0.10	387.45 ± 0.21
	10^{-6}	0.793 ± 0.05	6.55 ± 0.66	338.19± 0.13
150	0	1.47 ± 0.06	2.90 ± 0.04	356.42 ± 0.15
	10^{-4}	1.32 ± 0.01	3.02 ± 0.02	446.64 ± 0.17
	10^{-5}	1.16 ± 0.01	3.48 ± 0.05	464.13 ± 0.29
	10^{-6}	1.17 ± 0.02	3.28 ± 0.05	432.21 ± 0.12
F value		371.306	11933.32	3281.99
Salinity		20.945	799.893	88.771
Treatment		4.463	61.187	5.660
Salinity*Treatment				

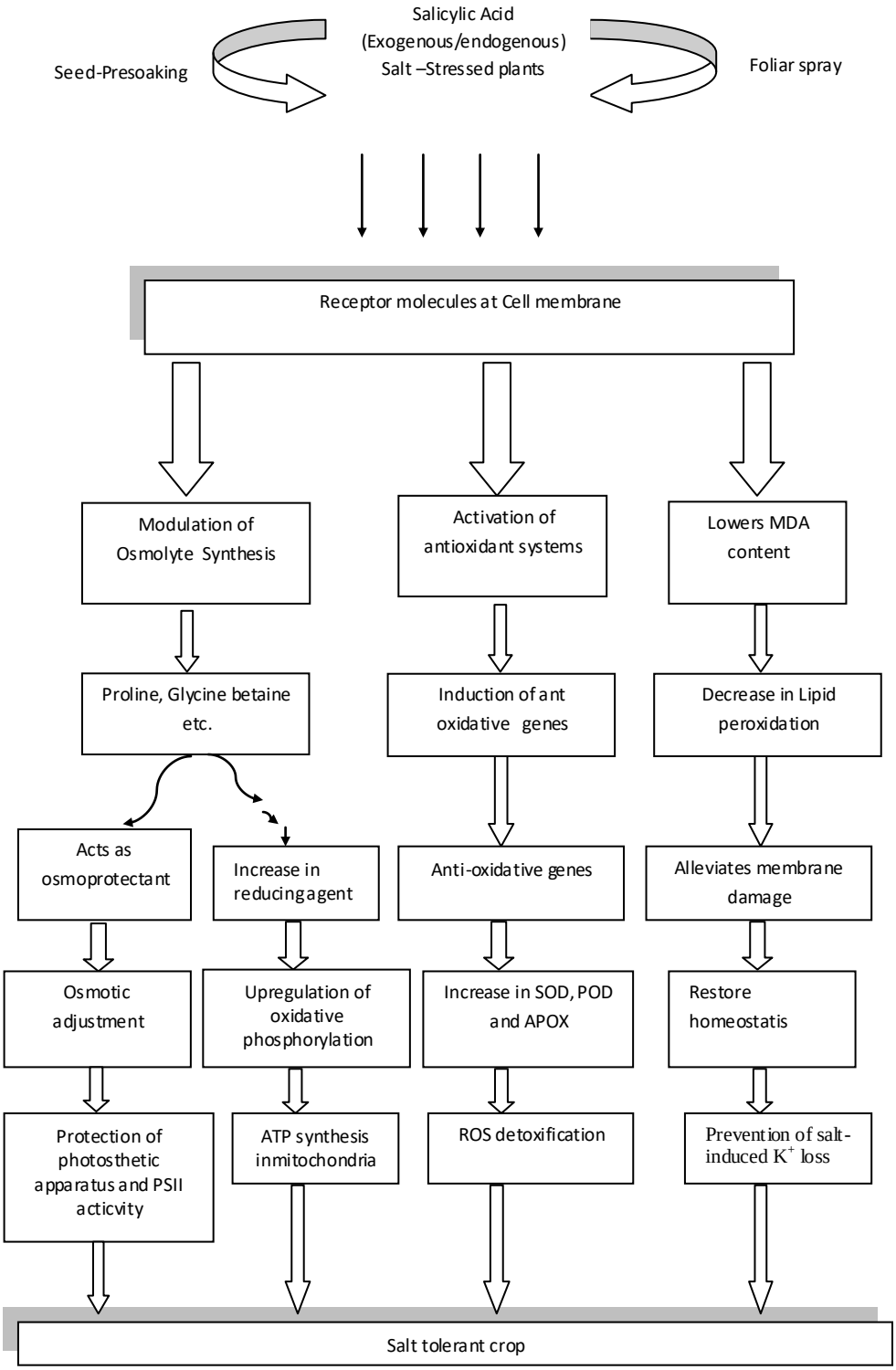

Figure 4: Possible Mechanism of Action of SA in Alleviating Salt Stress in Plants

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