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Amelioration of Salinity Stress Tolerance in Pea (*Pisum sativum* L.) by Exogenous Application of Salicylic Acid

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ABSTRACT

The research was aimed at evaluating physio-morphological and biochemical attributes of Pea (*Pisum sativum*) grown under salt (NaCl) stress and by exogenous application of salicylic acid. The experiment was laid out in petri-dishes and earthen pots with randomized complete blocks design having five replicates for each salt and salicylic acid treatment. The seeds grown in petri-dishes were primed with saline water (120 mM NaCl) and various levels of salicylic acid (0.1, 0.5, 1.0 mM). In the second phase of experimentation, certified variety of pea seeds was planted in earthen pots for 15 days and irrigation was carried out by tap water. After 15 days of growth, the seedlings are exposed to saline water treatment for further 60 days and exogenous applications of different concentration of salicylic acid were carried out in each group of plants simultaneously. Results obtained from this study indicate an increase in seed germination, shoot/root growth, fresh/dry mass, leaf area and diameter of plants by salicylic acid. Data obtained strongly suggest that induction of antioxidant defense is an important tolerance mechanism of pea plant to salt-stress. Salicylic acid improved growth of pea plants by regulating various biochemical attributes vis., proteins and proline contents and antioxidant enzymes activities. From commercial view-point, to induce plant resistance against both biotic and abiotic stresses, low levels of salicylic acid might strongly be suggested to enhance plant growth and productivity in pea.
Key Words: antioxidant enzymes, *Pisum sativum,* protein, proline, salicylic acid, salinity.

INTRODUCTION

Salinity refers to the deposition of soluble salts in soil to a limit that affects the agricultural and ecological wellbeing **(**Rengasamy, 2006). Soil salinity is considered among the main causes of soil degradation. All over the world, salinity has severely damaged approximately 30 % of the inundated and 6 % of total land (Chaves *et al.,* 2009) which resulted in financial loss of 12 billion US$ in agribusiness (Shabala, 2013). Approximately 19.5% of the cultivated area and 2.1% of the dry land farming on the earth is adversely affected by soil salinity (FAO, 2000). In Pakistan, 6.67 million hectares of land is severely harmed by salinity out of a total of 20 million hectares (Khan *et al.*, 2006). Salinity affects the soil porosity and by reducing water potential it causes drought conditions. High salt stress causes critical decrease in growth parameters like leaf area, root and shoot dry weights, stem elongation and root extension (Ashrafuzzaman *et al.*, 2002). Salinity is a major ecological issue that causes decrease in plant efficiency (Irshad *et al.,* 2002). Many researchers have provided details of changing levels of physiological and biochemical parameters produced by salt stress. The principal issue with agricultural products in saline conditions is reduction in plant growth due to osmosis (Song *et al.,* 2009). Salinity, as a major abiotic stress affects crop species adversely, disturbs homeostasis water potential, ion distribution and instigates restraint of growth and oxidative changes as an alternative stress. In spite of all this salinity leads to the creation and accumulation of reactive oxygen species in plants (Erdal *et al.,* 2011). Higher plant cells have advanced enzymatic and non-enzymatic antioxidant defense mechanism to lessen Reactive Oxygen Species (ROS) accumulation and oxidative damages by detoxifying free radicals (Borsani *et al.*, 2001). Reactive oxygen species truly upset metabolic system by producing oxidative damage to cell lipids, proteins and specifically nucleic acid (Rout & Shaw 2001). In plant cells treated under different stresses, initial steps take place mostly in apoplastic pathways (Atici & Nalbantoglu 2003).

Pea (*Pisum sativum* L.) is amongst the most significant vegetables in the world. It acts as a rich source of proteins, sugars and supplements for human diet. It is cultivated globally for its uses as human diet and fodder. It is considered third most significant legume vegetable after soybean and common beans (Timmerman-Vaughan *et al.*, 2005). Its pods have relatively large amount of protein and carbohydrates. It is thought to be as one of the most important sources of man diet globally (Hussein *et al.,* 2006). It is utilized as a fresh vegetable, frozen or canned. According to FAO (2000) information,

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around 12.2 million tons of pea productions were accomplished in 6.3 million hectors agricultural lands of the world with a normal yield of 1.930 kg per hector (http://www.fao.org/, Anonymous, 2007). Trypsin inhibitors found in pea are 5 to 20% less than in soybean (Kent & Endres, 2003). As a legume vegetable crop, pea has the capacity to fix 50-150 kg ha^{-1} nitrogen from air (Ozdemir, 2006). In spite of the fact that it is an important vegetable, its yield is somewhat low for quite a while. In Pakistan the average fresh pea production was 596 Kg/ha during 2001 (http://www.pakistan.goov.pk/food-division/publications, Anonymous, 2002). The pea cultivars developed by the vegetable growers in Pakistan produce fewer yields and their standard does not match with the cultivars grown in other world because of several constrains e.g., traditional agricultural practices, non availability of quality seed and soil salinity. Numerous experiments have been carried out to increase growth and yield of leguminous vegetables (pea and lupin) and cereals (Sandana, *et al.*, 2009).

Increase in salt stress resistance using salicylic acid (SA) was observed in maize (Hussein *et al.*, 2007), tomato (Shahba *et al.*, 2010) and mungbean plants (Nazar *et al.*, 2011). This effect of SA in plants is not consistent and it varies depending upon plant species as well as varieties, growth and developmental conditions and climatic factors as well (El-Mergawi & Abdel-Wahed, 2004). Small quantity of SA has been reported to increase growth of maize and wheat by Khan *et al*. (2003). It is observed that SA maintains plant growth and development as well as interacts with other defense mechanisms to different kinds of stresses (Bastam *et al.*, 2013). It has also been involved in glycolysis and plays important role in flowering, fruit production, uptake of ions, photosynthesis, opening and closing of stomata, evapotranspiration rate, temperature and heat resistance, nodulation and senescence (Khan *et al.*, 2003). Major function of salicylic acid is to act as regulator in response to various biotic stresses, but many researchers are now of the view that salicylic acid is also found to play role in many abiotic stresses like salinity stress resistance (Fahad & Bano 2012; Iqbal *et al.*, 2014; Fahad *et al.*, 2014). The present research work was planned with the aim to determine the biochemical, morphological and physiological changes under salinity stress in pea and to determine the potential effect of SA for the reduction of salinity stress in pea.

MATERIALS AND METHODS

A: Plant Materials and Experimental Conditions

For present work, certified Meteor variety of pea seeds, were procured from the market. The experimental set-up was installed in a field at University of Education Lahore, Okara Campus, Punjab, Pakistan in the month of October - November 2014. Seedling experiment was done at plant tissue culture laboratory. An average temperature for the experiment was 27 ±2 °C and relative humidity was around 64%. The experiment was performed under complete natural environmental conditions.

B: Seed Germination Study

Intact seeds, which were homogeneous and indistinguishable in size and colour, free from wrinkles and disease symptoms, were picked. Five pea seeds were sown in each of the five petri-dishes. Finally the petridishes were divided into 5 groups vis., A- Control (non-primed seeds under control conditions). B- seeds primed with 120mM salt only C- Primed seeds with 0.1 mM SA and 120mM salt, D- Primed seeds with 0.5 mM SA and 120mM salt, E: Seeds primed with 1.0 mM SA and 120mM salt.

C: Experimental Design

The pots used were 45 cm in diameter and 55 cm high. These were washed with tap water before experimentation. The soil was taken from field containing equal amount of Bhal and farmyard manure by weight. The potting mixture was crumbled into fine particles, mixed and sieved appropriately to homogenize it. Five seeds were sown in each pot and were left to grow under natural environmental condition. The experiment was conducted in completely randomized design (CRD) in three blocks where each block consisted of three replicates for each concentration. All pots in experiments were given tap water for fifteen days. Then plants were treated with different concentrations of SA through foliar spray. Taween twenty (20) was applied as surfactant for absorption of SA in the leaf tissues. Each block was divided in five groups as in case of seed germination experiment. The treatment was given for sixty days. Physical properties of potting mixture like pH, electrical conductivity (EC) and total dissolved solid (TDS) were recorded by pH meter and EC meter separately.

D: Harvesting and measurements of morphological parameters

The pot-grown-plants were harvested after sixty days of salt and salicylic acid treatment and various morphological parameters like shoot/root length, diameter, fresh/dry weight, leaf area etc.

were determined. Measuring tape was used for the measurement of shoot length of each plant and for determination of plants diameter, vernier caliper was used. Image J was used to find out the leaf area per plant (Rosband., image J, U. S. National Institute of Heath, Bethesa, Maryland, USA, http:/rsb.info.nihigov/IJ/.1997-2008).

E: Biochemical analysis

a. Extraction and estimation of total soluble protein and antioxidant enzyme activities:

Fresh leaves (ca. 0.5 g) were frozen in liquid N_2 and were grinded by using pestle and mortar. The powdered leaves were dissolved in 1 ml of 0.1M phosphate buffer (pH 7.2), 0.1 g polyvinyl polypyrollidone (PVP) and 0.5 % (v/v) Tritone X-100. The ratio of leaf tissue: buffer was kept at 1:2 (w/v) and then the homogenate was centrifuged at 14,000 rpm at 4°C for 30 minutes. The supernatant was stored at 0°C and used for quantitative estimation of proteins and antioxidant enzymes. For the estimation of protein contents, the Biuret method with certain modifications was employed (Racusen & Johnstone, 1961). Spectrophotometer (U-1100) was used to measure the optical density at 545 nm. The protein concentration was determined by the use of standard curve, Bovine Serum Albumin was used to prepare this curve. The method of Racusen and Foote (1965) with some modifications was used to analyze peroxidases. For superoxide dismutase activity, method proposed by Maral *et al*. (1977) was used which is based on measuring the capability of SOD to hinder the photochemical reduction of nitroblue tetrazolium.

b: Quantitative assay of proline

Proline contents were estimated by following method of Bates *et al*. (1973). Fresh leaves frozen in liquid nitrogen (0.5g) were grinded with 3% sulfosalicylic acid (10 ml), and centrifuged at 13000 rpm and 4ºC for 15 minutes. The extract (2 ml) was mixed with 2 ml acid ninhydrin and glacial acetic acid (2 ml) and incubated at100°C for an hour and then cooled at room temperature. After cooling, toluene (4 ml) was added and mixed with shaking. This sample was allowed to stand for 10 minutes to separate toluene and aqueous phases. The upper toluene layer was pipetted out carefully and its absorbance was recorded at 520 nm using toluene as a blank. The proline content was estimated by using standard curve.

The data recorded was subjected to ANOVA analysis and the means were separated by Duncan Multiple Range Test (DMRT) where there were differences at 0.05% probability level using SPSS computer pragramme (version 12.0.0).

RESULTS

A. Effect of seed priming with salicylic acid on % seed germination, shoot /root length

The effect of salicylic acid was seen on percentage rate of germination when seeds were primed with different concentrations of salicylic acid and 120 mM salt. 50.32% seeds sprouted under saline conditions and an increase in rate of germination was recorded as 63.68%, 69.49% and 74.58% when seeds were inundated with 0.1, 0.5 and 1.0 mM salicylic acid, respectively. Maximum growth (96.4%) was seen in seeds grown under control conditions.

A dynamic increase in shoot length was seen after the use of salicylic acid. The result showed that pea seedlings were severely influenced by salt stress, with decrease in shoot length from 3.9cm to 0.8cm under control conditions. The antagonistic impacts of salinity were decreased by salicylic acid. The shoot lengths recorded were 2.1, 3.1 and 3.6 cm with 0.1, 0.5 and 1.0 mM concentration of salicylic acid, respectively (Table I).

A steady increase in root length was recorded with increase of salicylic acid concentration under salt stress. As in case of shoot, the most prominent increase in root length was seen at 1.0mM salicylic acid concentration under saline conditions that was 5.7cm. Other concentrations observed were viable on the basis that root lengths recorded 4.5cm for 0.1mM and 4.8cm for 0.5 mM when compared with 1.7cm (120 mM NaCl). The information about effect of salicylic acid under salinity stress in pea seeds is shown in table I on germination rate, shoot length, and root length of seedlings.

B. Effect of SA spray on shoot length/ diameter and leaf area of pea plant

Increase in shoot length and diameter was observed with an increase in salicylic acid concentration. The highest increase was recorded at 1.0 mM, SA concentration. Average length of shoot of salt treated, control plants was 17.45cm which increased to 28.49 cm when 1mM SA treatment was applied. The shoot diameter was 3.78, 4.23 and 5.28 mm when 120 mM salt was applied with SA at 0.1, 0.5 and 1.0 mM concentrations, respectively.

The leaf area increased steadily with increasing levels of salicylic acid under both control and saline condition. At 0 mM NaCl concentration leaf area was 62 cm^2 and at 120 mM salt, leaf area was decreased up to 32 cm^2. In salt treated plants when foliarily was sprayed with salicylic acid (1.0

mM) the leaf area increased from 63 to 74 cm^2 (Table II).

C. Effect of salicylic acid spray on fresh / dry mass of shoot and root

The foliar spray of salicylic acid substantially alleviated the salinity enforced growth inhibition in terms of biomass production. Under salinity stress (120 mM NaCl), all the three concentrations of SA (0.1, 0.5 and 1.0 mM) were found to be almost equally good in amelioration of salt stress in terms of increasing shoot fresh weight of plants. However, a maximum increase in shoot dry weight was observed when salicylic acid was applied at the concentration of 1.0 mM.

It was observed that the foliar spray of salicylic acid showed little effect in alleviating the negative effects of salinity on root fresh/dry weights and mostly led to significant decrease at all the tested concentrations (Table III). Root biomass was not much improved after foliar spray of salicylic acid alone as compared to their corresponding control.

D. Effect of salicylic acid spray on protein contents of pea plants

The soluble protein contents of plants demonstrated an increase after foliar spray of SA at 0 mM NaCl concentration. In any case, this increase was recorded just at 0.1 and 0.5 mM SA concentrations. A most extreme increase (0.29 mg/g tissue) was seen at 0.5 mM salicylic acid concentration. Usage of salt stress brought about increased in soluble protein contents while SA application created a reduction in soluble protein content in pea plants. The soluble protein contents were 0.32, 0.29 and 0.33 mg/g tissue after 0.1, 0.5 and 1.0 mM SA spray, respectively.

E. Effect of salicylic acid spray on peroxidase and SOD activity of pea plants

The peroxidase activity of plants sprayed foliary with 0.1 or 0.5 mM salicylic acid concentrations was precisely the same (0.14 mg/g tissue) which demonstrated a reduction to 0.14 mg/g tissue or 0.13 mg/g tissue at 1.0 mM salicylic acid concentration applied alone or in combination with salt. It was observed that 1.0 mM salicylic acid concentration ended up being less compelling in mitigating negative impacts of salinity.

Table IV demonstrates that the SA application at 0 mM NaCl concentration basically did not enhance the superoxide dismutase activity when compared with the control. If there should be an occurrence of foliar spray under salt stress, 0.1 mM SA concentration brought about an increase of SOD activity. On the other hand, an increase up to 96.58 and 78.47 U/mg proteins from 64.26 U/mg proteins was acquired at 0.5 mM and 1.0 mM SA concentration, respectively. It is apparent from table that increase in SOD activity was more pronounced under salicylic acid treatment through foliar application.

F. Effect of foliar spray of Salicylic acid on proline contents of pea plants:

The proline content of pea plants was influenced by foliar spray of salicylic acid. The most astounding proline contents (47.86 µmol/g F.W) were observed at 120 mM NaCl concentration after spray with SA at the concentration of 1.0 mM. This is followed by 37.99 µmol/g dry weight in salinized plants when foliary sprayed with 0.5 mM SA. At 0 mM NaCl concentration the proline contents were enhanced to 47.86µmol/g from 13.89 µmol/g (proline contents of control plants) after salicylic acid (1.0 mM) application. Under salt stress, the proline contents were 29.15 and 37.99µmol/g after foliar spray with SA at 0.1 and 0.5mM concentrations, respectively.

Table I: Effect of SA on seed % germination and shoot/ root length

Treatments	Germination (%age)	Shoot length (cm)	Root length (cm)
Control	96.4 ± 2.03^{a}	3.9 ± 1.07^{a}	6.8 ± 1.83^{a}
Salt 120mM	50.32 ± 1.10^{d}	0.8 ± 0.03^{d}	1.7 ± 0.93^{d}
S+SA 0.1mM	63.68 ± 1.92^{cd}	2.1 ± 0.01^{c}	4.5 ± 1.03^{c}
S+SA 0.5mM	69.49 ± 1.33^{c}	3.1 ± 0.93^{b}	4.8 ± 1.90^{c}
S+SA 1.0mM	74.58 ± 1.08^{b}	3.6 ± 0.83^{ab}	5.7 ± 1.09^{ab}
Significance	*	*	*

The results are based on 5 replicates for each treatment.
The ± sign represents the Standard Error.
(*) Significant or (NS) non-significant at 0.05 %.

Table II: Effect of foliar spray of SA on shoot length/ diameter and leaf area

Treatments	Shoot length (cm)	Shoot diameter (mm)	Leaf area (cm^2)
Control	30.33± 4.50[a]	5.47 ± 0.97[a]	74 ± 26.62[a]
Salt (120 mM)	17.45 ± 1.35[c]	3.12 ± 0.25[b]	32 ± 8.53[d]
S+ SA (0.1 mM)	19.79 ± 3.85[c]	3.78 ± 0.40[b]	38 ± 11.51[c]
S+ SA (0.5 mM)	23.65 ± 1.31[b]	4.23 ± 0.28[ab]	51 ± 15.28[b]
S+ SA (1.0 mM)	28.49 ± 2.32[a]	5.28 ± 0.32[a]	63 ± 12.26[ab]
Significance	*	*	*

The results are based on 5 replicates for each treatment.
The ± sign represents the Standard Error.
(*) Significant or (NS) non-significant at 0.05 %.

Table III: Effect of foliar spray of salicylic acid on biomass production

Treatments	Shoot weight (g)		Root weight (g)	
	Fresh wt. (g)	Dry wt. (g)	Fresh wt. (g)	Dry wt. (g)
Control	12.53 ± 2.93[a]	2.35 ± 0.48[a]	5.89 ± 2.62[a]	1.78 ± 0.44[a]
Salt (100 mM)	09.63 ± 0.92[c]	1.70 ± 0.23[d]	1.14 ± 2.24[e]	0.94 ± 0.32[e]
S+ SA (0.1 mM)	10.86 ± 0.83[b]	1.84 ± 0.17[d]	2.85 ± 1.17[d]	1.07 ± 0.22[d]
S+ SA (0.5 mM)	11.15 ± 1.59[b]	2.01 ± 0.34[c]	3.17 ± 0.79[c]	1.21 ± 0.15[c]
S+ SA (1.0 mM)	12.04 ± 0.94[a]	2.18 ± 0.22[b]	4.38 ± 0.65[b]	1.52 ± 0.22[b]
Significance	*	*	*	*

The results are based on 5 replicates for each treatment.
The ± sign represents the Standard Error.
(*) Significant at 0.05 %

Table VI: Effect of foliar spray of Salicylic acid on soluble protein contents, Peroxidase, Superoxide dismutase activities and proline contents of salt-stressed pea plants.

Treatments	Soluble Protein Contents (mg/g tissue)	Peroxidase activity (mg/g tissue)	SOD activity (U/mg protein)	Proline Contents (µ mol/g FW)
Control	0.30 ± 0.004^{b}	0.13 ± 0.008^{b}	50.47 ± 11.36^{e}	13.89 ± 0.639^{e}
Salt (120 mM)	0.38 ± 0.012^{a}	0.11 ± 0.006^{b}	64.26 ± 7.143^{d}	22.04 ± 2.676^{d}
S+ SA (0.1 mM)	0.32 ± 0.011^{b}	0.14 ± 0.007^{a}	51.87 ± 10.25^{bc}	29.15 ± 5.008^{c}
S+ SA (0.5 mM)	0.29 ± 0.005^{c}	0.14 ± 0.007^{a}	96.58 ± 3.467^{a}	37.99 ± 5.719^{b}
S+ SA (1.0 mM)	0.33 ± 0.008^{b}	0.12 ± 0.007^{b}	78.47 ± 4.137^{c}	47.86 ± 4.822^{a}
Significance	NS	NS	*	*

The results are based on 5 replicates for each treatment.
The ± sign represents the Standard Error.
(*) Significant at 0.05% probability level.

DISCUSSION

Salt stress proves to be a strong inhibitor of plant growth and development as higher levels of salt stress are found to produce ion imbalance and osmotic stress in different crops (Maggio *et al*., 2000). These impacts, in turn can produce different kinds of stress like oxidative damage that may end up in decreased growth of plants (Zhu, 2001). Various methods are being exercised by scientists and researchers in different plants to ameliorate the adverse effects of salinity. Seed priming is considered as very useful tool to enhance tolerance against salt stress in different crops. Present study revealed that presoaking of seeds with salt (120 mM) reduced the germination percentage significantly (50.32 %) as compared to control (96.4%). Seed germination is an important phase of the plant for further growth. However, in case of SA treated seeds, germination %age was 63.68, 69.49 and 74.58% at 0.1, 0.5 and 1.0 mM SA, respectively. Maximum increase in germination %age was recorded at 1.0 mM SA. Shoot and root length of these presoaked seeds were increased from 0.8 to 3.6 cm and 1.7 to 5.7 cm at 1.0 mM salicylic acid concentration. The reduction in growth parameters might be due to the effects of salt on inhibition of cell division and elongation or due to the accumulation of Na^{+} in plant tissues which interferes with normal metabolism and creates an oxidative stress as reported by Sasikala & Prasad (1994). The priming of seeds by SA have increased germination rate under normal (Afzal *et al*., 2005) and under salt stress conditions (Hameed *et al*., 2010) because of its role in cell division and elongation. In line with these results, Keshavarzi *et al.* (2011) studied the impact of different concentrations of salt stress on seed germination of *Spinacia oleracea* L. they tested four different levels of salinity (0, 50, 100 and 150mM) and found that percentage germination, fresh and dry weight of shoot and root were badly affected by increasing salt concentration. The impact of seed priming with salicylic acid and acetylsalicylic acid to enhance seed germination and salinity resistance of hot pepper was also reported by Khan *et al,* (2009). In the present investigation, it was seen that as the level of SA was enhanced to more than 1.0 mM, in the medium, it resulted in reduction of growth of pea plant. The reason might be the adverse and harmful impact of SA application at higher concentrations. In previous studies the deleterious impact of higher salicylic acid concentrations (above 1.0 mM) was seen in bean and *Lycopersicum esculentum* when cultivated under higher and lower temperature stress (Senaratna *et al*., 2000). The data

demonstrates that salicylic acid stimulates abiotic stress resistance in different plant cultivars by maintaining the expression of many receptor protein kinases which are reported to stimulate responses to particular stress signals, for example, after wounding in *Brassica oleracea* (Pastuglia *et al*., 1997) or in peaches (Bassett *et al*., 2005).

The harmful effect of salt was also found in present investigation in case of pot experiment, where use of high salt levels (120 mM of NaCl) to pea plants deleteriously affected many growth (stem and root length, number of nodes, fresh/dry weights) and biochemical (protein and antioxidant enzymes) parameters. Similar results were reported by various authors who worked on different pea varieties and other plant species treated with different concentrations of salt (Benavides *et al*., 2000; Rashid *et al*., 1999). In this investigation, pea plants when treated with different levels of (0.1, 0.5 and 1.0 mM) of SA exogenously under salt stress showed a remarkable improvement in growth and development. Shoot length was increased from 17.45 to 28.49 cm, shoot diameter from 3.12 to 5.28 mm and leaf area from 32 to 63 cm^2 at 1.0 mM salicylic acid concentration. Similarly fresh/dry weight was also increased significantly by exogenous application of SA. Shoot fresh weight increased from 9.63 to 12.04 g at 1 mM SA concentration and dry weight from 1.70 to 2.18g. Similarly root fresh weight increases from 1.14 to 4.38 g and dry weight from 0.94 to 1.52 g respectively. In both cases maximum increase was observed when 1 mM SA was applied exogenously to salt stress plants. The increase in biomass at above mentioned concentrations might be due to increased shoot length and fresh weight. Whereas, SA through irrigation and as foliar spray may exert little effect on the bean plants under normal growth conditions (Palma *et al.,* 2009).

In this study, when the salt treated plants were supplied SA (1.0 mM) exogenously protein contents decreased significantly. Similar results were reported earlier that quantity of total soluble protein (TSP) increased at high salinity and decreased at low salinity. Biochemical strategies to cope with salt stress in plants include osmotic adjustment by accumulation of compatible inorganic as well as organic solutes and an efficient enzymatic and non-enzymatic antioxidant system (Sairam & Tyagi, 2004) are well proven in literature. Desoky & Merwad (2015) studied the interacting impact of foliar use of antioxidants i.e., ascorbic acid and salicylic acid and various concentrations of salt stress on protein contents of *Triticum aestivum.* A higher level of proline contents were also found after foliar application of salicylic acid in this study. Hussein *et al*. (2007) reported same results on *Zea mays* by foliar application of salicylic acid where an enhanced level of proline was found under salt stress. While in contrast to these results Fidalgo *et al.* (2007) reported a decreased protein contents in potato at 100 and 200 mM salt under glass-house. This decrease in TSP might be due to the higher salt levels that plays a role in osmotic imbalance and in turn causes reduction in water uptake by the plants. The high protein in response to salt stress may act as an osmolyte in maintaining osmotic balance (Sajid & Aftab, 2009). So it is considered that SA treatments may stimulate hydrolysis of protein providing an osmolytes (proline) for stress-tolerance. The proline accumulation by SA application in normal and salt-stressed plants can be considered as an important biomarker involved in SA induced protective mechanism for stabilization of essential proteins and protection of enzymes.

In present investigation specific activity of POD, SOD to scavenge H_2O_2 (ROS) represents an overall increasing trend at the tested SA levels. However, maximum increase in the activities of both studied enzymes was recorded at 0.5 mM SA instead of 1.0 mM . This increase in antioxidant enzymes activity at lower concentration of SA and decrease at higher concentrations were determined by El-Tayeb, (2005)). High concentrations of SA or prolonged effects of SA, directly on indirectly through the formation of certain complexes in medium may decrease the POD and SOD activity. Which in turn multiply oxidative stress (reactive oxygen species: ROS) by disrupting balance between H_2O_2 generation and its scavenging system as reported by Horvath *et al.* (2007); Senaratna *et al.* (2000); Haider & Saifullah, (2000).

Conclusion: SA pretreatment confers salt resistance, and is likely to prepare the plants for forthcoming oxidative stress by increasing antioxidant enzymes activity and proline at the expense of protein degradation. Such types of ameliorative impact of SA on growth of plants grown under saline conditions might be due to the reason that salicylic acid stimulates the formation of ROS and enhances the generation of hydrogen peroxide in plants that as a result decreases the oxidative harm caused by salt stress. It is concluded from the study that salicylic acid treatment especially of 1.0 mM proved very useful in improving growth and ameliorating the adverse effects of salt stress.

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