Pak. J. Bot., 51(5): 1551-1559, 2019. DOI: http://dx.doi.org/10.30848/PJB2019-5(12)

SALICYLIC ACID ALLEVIATES SALINITY STRESS THROUGH THE MODULATION OF BIOCHEMICAL ATTRIBUTES AND SOME KEY ANTIOXIDANTS IN WHEAT SEEDLINGS

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Abstract

A study was conducted to evaluate the role of salicylic acid (SA; 0, 0.5 and 0.75 mM) on the growth and activity of antioxidant enzymes and biochemical attributes in wheat (*Triticum aestivum* L.cv. Sods 1) under salinity stress. Salinity exposure (0, 25, 75 and 125 mM NaCl) reduced growth of wheat significantly by reducing the fresh and dry weight of shoot and root, leaf development and inducing necrosis on old leaves. Lipid peroxidation and production of hydrogen peroxide (H_2O_2) increased by 3.37 fold and 2.54 fold, respectively, while membrane stability declined with 125 mM NaCl concentration which were however, ameliorated by the application of SA. Under normal conditions, application of SA (0.5 mM) improved growth significantly compared to the untreated controls. Salinity (125 mM) stress enhanced the accumulation of proline (4.63 fold), carbohydrates (39.61%), free amino acids (9.44) and protein content (7.91%) which were further stimulated by application of SA leading to better stress adaptation. Application of SA to salinity stressed upregulated the activity of antioxidant enzymes like SOD, CAT and APX by 1.76 fold, 2.25 fold and 2.22 fold, respectively leading to better elimination of reactive oxygen species and protection against oxidative stress. Moreover, excess uptake of Na in salinity stressed plants reduced the uptake of K^+ and initiated leaf necrosis. However, application of SA mitigated these negative effects to considerable extent. In conclusion, salinity stress adversely affected the growth and development of wheat plants. However, supplementation of proper dosage of SA mitigated these negative effects of salinity through the modulation of the levels of osmolytes, activities of antioxidant enzymes and uptake of essential elements.

Key words: Wheat; Salicylic acid; Necrosis; Antioxidant enzymes; Lipid peroxidation; Proline; Salinity.

Introduction

Plant growth and development is affected by high salt concentrations in soils which ultimately cause threat to food production globally (Ashraf & Foolad, 2007). Approximately 5% of arable land is adversely affected by high salt concentrations posing threat to global food security (Ghassemi *et al.*, 1995). Iqbal *et al.,* (2018) described the impacts of salinity stress on numerous characteristics of wheat crop including morphological, physiological, biochemical, molecular and anatomical aspects. The problems experienced by higher plants owing to salt stress result from osmotic stress and ionic stress resulting from high concentrations of toxic ions exceeding the threshold to which most plants are adapted. Ahmad *et al.,* (2017) also reported reduced growth, decrease in pigment content and uptake of essential elements in *Pisum sativum* under NaCl stress. Reduction in pigment synthesis and alteration in nutrition assimilation under salt stress directly affects photosynthetic efficiency, plant-water status, enzyme activities and synthesis of proteins and carbohydrates and the stability of cell membranes (Alamgir *et al.*, 2008; Türkan & Demiral, 2009; Alzahrani *et al.,* 2019). Generation of reactive oxygen species (ROSs) in plants is also the outcome of salinity stress (Ahmad *et al.*, 2014; Ahmad *et al.*, 2015; Ahmad *et al.*, 2016; Ahmad *et al.*, 2017; Ahmad *et al.*, 2018b). ROS causes oxidative damage because of their high affinity to react with biomolecules like DNA, RNA, proteins, lipid membranes etc and alter their structural and functional ability (Heidari & Golpayegani, 2012). Plants growing in saline environments have various strategies at both the whole-plant and cellular levels allowing them to overcome salinity stress. Osmotic stress is regulated by the enhanced levels of osmolytes like proline, protein, carbohydrates and amino acids (Ahmad *et al.*, 2014; Ahmad *et al.*, 2015; Ahmad *et al.*, 2016; Ahmad *et al.*, 2017; Ahmad *et al.*, 2018b). The oxidative stress is managed by the regulation of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) etc (Eyidogan & Öz, 2007).

Higher plants have evolved multiple genetically and physiologically complex strategies and mechanisms, affecting numerous plant processes at all levels of plant cells, to prevent Na^+ compartmentalization in leaf cells as well as to overcome the dominating and persistent osmotic stress (Schubert *et al.*, 2009). Since Na^+ translocation to the shoot occurs via the xylem, driven by transpiration, the main site for Na^+ toxicity in most plants is the shoot and its exclusion from the shoot grant salt tolerance in plants (Munns & Tester, 2008).

Alleviation of abiotic stress by the application of salicylic acid (SA) has been reported by many workers (Ahmad *et al.*, 2011; Ahmad *et al.*, 2018b). Ma *et al.*, (2017) reported that the exogenous SA effectively improved the growth, photosynthesis, antioxidant enzyme activity, stoma and chloroplast development of

D. superbus. Salicylic acid activates carotenoid synthesis and enhances the rate of de-epoxidation in wheat under salt stress (Moharekar *et al.,* 2003). Earlier it has been evidenced that SA strengthens antioxidant system in *Brassica juncea* (Yusuf *et al.,* 2008).Wheat is an important cereal crop in many parts of the world. Around 36% population of the world uses wheat as staple food as it contains 55% carbohydrates (Hasanuzzaman *et al.,* 2017). Wheat is often susceptible to salt stress due to which its yearly production is decreasing (Ashraf & Foolad, 2007). Plant scientists are searching for some sustainable approaches that could escalate the production in salt affected soils. Phytohormones (SA) could be one of the approaches that will suffice the need and has been reported earlier also (Ahmad *et al.,* 2018a; Bali *et al.,* 2018; Mir *et al.,* 2018). Thus, the present study was designed to evaluate the effect of different concentrations of NaCl on growth and biomass yield, osmolytes, activity of enzymatic antioxidants and mineral uptake. Also the role of SA in alleviating the NaCl toxicity through the modifications of above key parameters in wheat plants is also ascertained.

Materials and Methods

Growth experiment: Sterilized seeds of wheat (*Triticum aestivum* L. cv. Sids1) were sown in 30 cm diameter plastic pots containing clay soil and were maintained in a greenhouse under natural conditions. All pots were irrigated with double distilled water (DDW) until the appearance of the fourth true leaves. Thereafter plants were treated with different salinity levels (0, 25, 75, and 125 mM NaCl), with and without salicylic acid (SA) with different concentrations (0, 0.5 and 0.75 mM). SA was exogenously sprayed at the rate of 20 ml per pot and the control plants were provided DDW only with same quantity. After 27 days of growth plants were uprooted and weighed for fresh and dry weight. For dry weight plant material was kept in oven at 105°C for 24 hours.

Estimation of free proline, free amino acids, carbohydrates and total protein: The method of Bates *et al.,* (1973) was employed for the estimation of proline content.

Free amino acid levels were determined according to method of Moore & Stein (1948).

The anthrone-sulfuric acid method of Fales (1951) and Schlegel (1956) was used for determining carbohydrate content. A calibration curve using pure glucose was developed and expressed as mg glucose g^{-1} DW.

The method of Lowry *et al.,* (1951) was used for the estimation of total protein.

Estimation of hydrogen peroxide (H_2O_2), Lipid peroxidation (MDA) and Electrolyte leakage (EL): The method of Mukherjee & Choudhuri (1983) was used for the estimation of H_2O_2 and Rao & Sresty (2000) for malondialdehyde (MDA) content. The protocol of Dionisio-Sese & Tobita (1998) was used for determination of electrolyte leakage.

Assay of antioxidants

A leaf sample of 5 mg was homogenized in 100 mM Tris-HCl (pH 7.5) containing DTT (5 mM), $MgCl_2$ (10 mM), EDTA (1 mM), magnesium acetate (5 mM), PVP-40 (1.5 %), PMSF (1 mM) and aproptinin (1 μg mL^{-1}). The material after crushing was allowed to pass through thin cloth and then centrifuged at 4°C at 10,000 rpm for 15 min. The sample collected was used as enzyme source.

The protocols of van Rossum *et al.,* (1997), (Aebi, 1984) and Nakano & Asada (1981) were used for the determination of activity of superoxide dismutase (EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX, 1.11.1.11).

Element analysis: Ground plant material was used for the estimation of Na^+ and K^+, 200 mg of dried plant material (shoots and roots) was mixed with 20 ml of concentrated H_2SO_4 and perchloric acid on a hot plate for 2h. Solution was filtered through a filter paper into 50 mL volumetric flasks and volume makeup was done with distilled water. The concentrations of Na^+ and K^+ were measured using a flame photometer (model M7D).

Statistical analysis

The data was statistically analyzed by one-way ANOVA by SPSS 13.0. Multiple comparisons, separating means in homogeneous subgroups, were conducted using Tukey's post-hoc test.

Results and Discussion

Effect of salinity and salicylic acid application on fresh and dry mass: Salinity stress (125 mM) decreased the shoot FW by 44.79% and DW by 49.13% over control plants. Supplementation of SA (0.50 mM) to NaCl (125 mM) treated plants enhanced the FW and DW of shoot by 30.68% and 32.88% respectively as compared to NaCl alone treated plants. SA (0.75 mM) decreased the shoot FW and DW as compared to control (Fig. 1A-B).

Reduction in plant biomass yield by NaCl stress was also observed by Ahmad *et al.,* (2015) in mustard, Ahmad *et al.,* (2014) in mulberry, Rasool *et al.,* (2013) in chickpea and Azooz *et al.,* (2011) in broad bean. Mohamed and Shaddad (2013) also reported the negative effect of salinity on the growth and yield of wheat under high salinity stress. High salt concentration reduced shoot growth, probably because of increased osmotic pressure or sodium ion concentration within leaf cell cytoplasm. Sodium ions could be a toxicity factor that causes reduction in shoot dry weights (Pitann *et al.,* 2013; Orsini *et al.,* 2012). Supplementation of SA enhanced the fresh and dry weight in the present study and the results confirm with the results of Iqbal *et al.,* (2006) in wheat cultivars. Gunes *et al.,* (2007) also observed that treatment of SA to maize plant showed higher dry weight than untreated plants grown under NaCl stress. Ma *et al.,* (2017) also reported that supplementation of SA enhanced the growth and biomass yield in *Dianthus superbus* under NaCl stress.

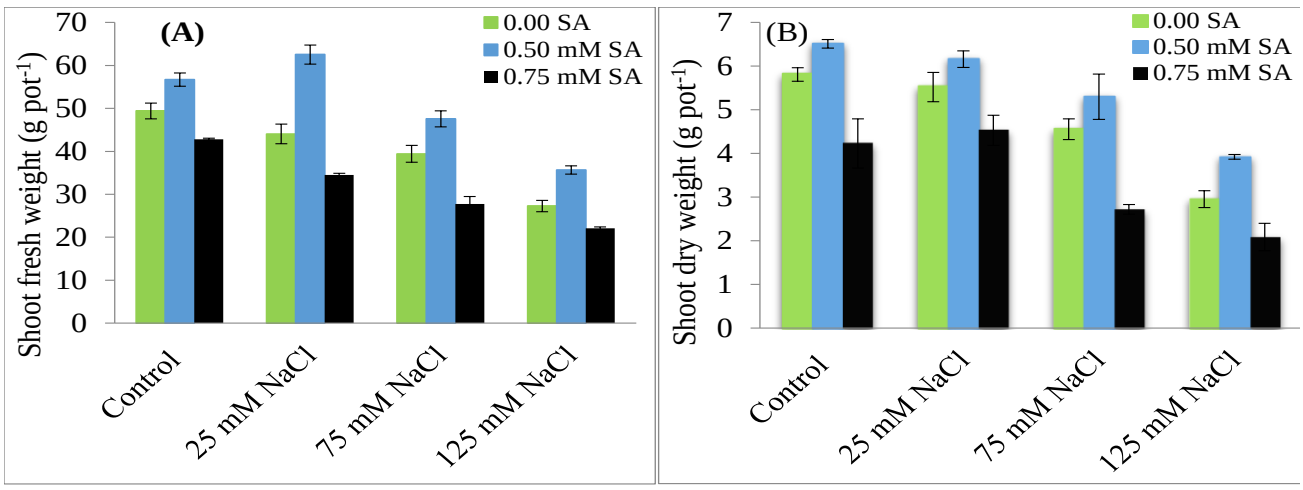

Fig. 1. Interactive effect of salicylic acid and different levels of NaCl on (A) fresh and (B) dry weight of shoot of wheat plants (g pot-1). Values are means ± SD of three independent replications (n = 5).

Effect of different salinity levels and exogenous SA application on H_2O_2, MDA content and EL: Salt stress enhanced H_2O_2, MDA content and EL at all stress levels as shown in Fig. 2A-C. The H_2O_2, MDA content and EL was increased by 2.54, 3.37 and 2.00 fold in NaCl (125 mM) treated plants in comparison to control plants. However, supplementation of SA (0.50 mM) to 125 mM NaCl treated plants declines the H_2O_2 content by 3.43 fold, MDA content by 1.80 fold and EL by 2.59 fold with respect to NaCl alone treated plants. The SA (0.75 mM) concentration elevates H_2O_2, MDA content and EL content more than NaCl treated plants alone.

The increased levels of H_2O_2, MDA content and EL validate the finding of Ahmad *et al.,* (2015) in *Brassica juncea*. NaCl stress increased the accumulation of H_2O_2 was also reported by Zhang *et al.,* (2014) in cotton Giannakoula and Ilias (2013) in tomato seedlings. The enhanced level of H_2O_2 might be due to decline in H_2O_2 scavenging activity (Senadheera *et al.*, 2012). It is believed that the H_2O_2 and MDA contents are the indicators of oxidative stress intensity in plants (Ahmad *et al.*, 2017a; Ahanger & Agarwal, 2017b). Salinity causes membrane damage via membrane lipid peroxidation (Mishra & Choudhuri, 1999) and our results also demonstrated a positive correlation between membrane permeability and MDA concentration. Increased generation of H_2O_2 accelerates the Haber-Weiss reaction resulting in increased membrane damage. Ahmad *et al.,* (2014) reported the elevated levels of MDA content under salt stress in mulberry thus enhancing electrolyte leakage. Saleem *et al.,* (2011) and Li (2009) also reported synergestic effect of NaCl and MDA accumulation in Okra and tomato respectively. Supplementation of SA decreased the H_2O_2, MDA accumulation and also electrolyte leakage in the present study. SA acts as an antioxidant and helps in scavenging the ROS like H_2O_2 under NaCl stress (Ahmad *et al.*, 2015). Accumulation of antioxidants like SOD, CAT and APX also helps in decreasing accumulation of H_2O_2 and MDA by quenching of ROS thus minimized the peroxidation of membranes (Ahmad *et al.*, 2015) and electrolyte leakage. Shen *et al.,* (2014) suggested that SA acted as a signalling molecule and activated different defensive mechanisms in plants. MDA levels in *Triticum aestivum* decreased significantly in response to salicylic acid treatment, which supports the suggestion that salicylic acid treatment can ameliorate stressful conditions by increasing membrane stability in *T. aestivum*.

SA application improves the synthesis of proline, free amino acids, carbohydrate and protein: Fig. 3 represents the results related to proline, free amino acids, total carbohydrates and protein content. NaCl (125 mM) enhanced the proline content by 4.63 fold as compared to control plants. Further enhancement in proline content in NaCl treated plants by 1.81 fold and 5.12 fold was observed with 0.50 mM and 0.75 mM SA respectively (Fig. 3A).

The higher NaCl content (125 mM) enhanced free amino acids, total carbohydrate and protein content by 9.44%, 39.61% and 7.91% respectively as compared to control (Fig. 3B-D). Application of SA (0.50 mM) enhanced the free amino acids content by 19.87%, total carbohydrate by 38.72% and protein content by 22.51% over NaCl alone treated plants.

Accumulation of proline under salt stress has been observed in *D. superbus* (Ma *et al.*, 2017); *Brassica juncea* (Ahmad *et al.*, 2015) and, *Lens culinaris* (Misra & Saxena, 2009). Proline enhancement in plants contributes to osmotic adjustment, maintains integrity of membranes (Misra & Saxena, 2009). Proline has having characteristics of antioxidants and helps in ROS quenching, thus mediates cell protection from oxidative damage (Jogaiah *et al.,* 2013; Ahmad *et al.,* 2010). Aggarwal *et al.,* (2012) reported that under NaCl stress proline played a role in energy storage i.e., C and N. The results related to enhanced levels of free amino acids in present study are consistent with the reports of Jaleel *et al.,* (2008), who have reported a varied accumulation of amino acid levels with different concentrations of NaCl stress. In this study, salicylic acid application mitigated the negative effects of NaCl toxicity on amino acid levels. In accordance with these observations, Palma *et al.,* (2013) stated that salicylic acid application via spraying enhanced the activity of the photosynthetic apparatus and enzymes in beans. In wheat, the amino acid levels were suggested to increase with increasing salicylic acid concentration (Khan *et al.*, 2013).

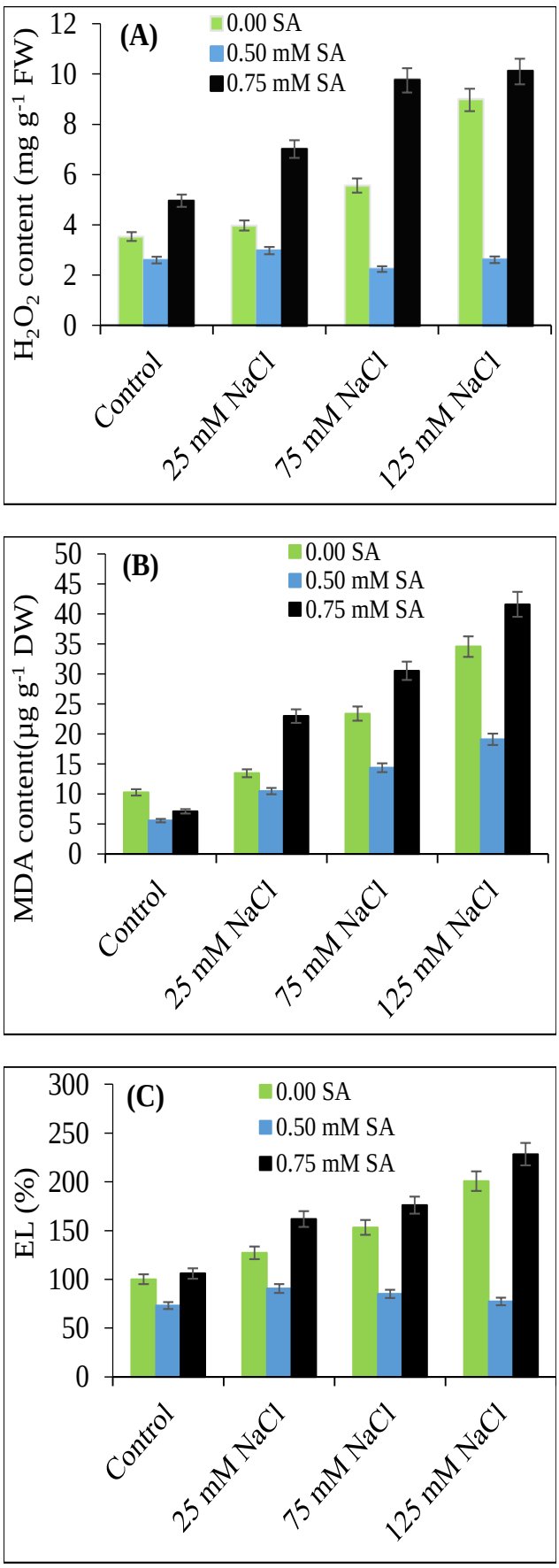

Fig. 2. Interactive effect of salicylic acid and different levels of NaCl on the (A) percentage Electrolyte leakage, (B) lipid peroxidation and (C) hydrogen peroxide in wheat plants. Values are means ± SD of three independent replications (n = 5).

Enhanced carbohydrate content under salt stress has been observed by Yin *et al.,* (2010) in *Solanum lycopersicum*. Hartzendorf & Rolletschek (2001) also reported that NaCl enhanced the carbohydrate content in *Phragmites australis*. Metabolism of carbohydrate is very sensitive to salinity stress (Oliver *et al.*, 1998).

Control as well as salt stressed plants when supplemented with SA significantly improved the accumulation of carbohydrates in the current study. Our observations are in accordance with the conclusions of Agamy *et al.,* (2013) in tomato. Naureen & Naqvi (2010) reported that increased carbohydrate levels under salt stress could be suitable parameters for identifying salt-tolerant wheat plants. This increase in total carbohydrate levels is a mechanism of osmoregulation (Siringam *et al.*, 2012; Shaddad *et al.*, 1990), and is involved in protecting plants from oxidative stress and maintaining the structures of proteins and membranes as well (Hajihashemi *et al.*, 2006).

The soluble proteins show elevation at lower concentrations of NaCl but at higher concentrations (125 mM) it showed a decline, in the current study. However, application of SA enhanced the accumulation of proteins. Proteins have been explained to play a great role on osmotic adjustment (Ashraf & Harris, 2004). Accumulated proteins acts as a storage form of nitrogen (Ahmad *et al.*, 2015).

Shoot and root Na^+ and K^+ and leaf necrosis: NaCl stress (125 mM) enhanced shoot Na^+ by 182.60% and root Na^+ by 1241.66% as compared to control plants. Supplementation of 0.50 mM SA decreased the accumulation of Na^+ by 26.15% in shoot and 14.90% in root as compared to NaCl alone treated plants (Table 1).

Shoot K^+ was decreased by 3.60% and root K^+ by 22.66% with 125 mM NaCl (125 mM) as compared to control (Table 1). SA (0.50 mM) enhanced shoot and root K^+ by 8.41% and 167.24% respectively as compared to NaCl alone treated plants. SA (0.75 mM) decreased the K^+ accumulation in both shoot and root under NaCl stress.

Excess accumulation of Na is considered to be toxic to plant growth while as K has an indispensable role in plant growth regulation due to its involvement in activities like protein synthesis, enzyme activation, osmoregulation etc. (Ahanger & Agarwal, 2017a; Ahanger *et al.*, 2017). Greater accumulation of K^+ leads to growth protection under stressful conditions by preventing oxidative damage to membranes and in the present study SA application improved the uptake and accumulation of K^+ probably by affecting the ion transport proteins involved in its uptake. SA prevented the excess accumulation of Na and mediated the exclusion and partitioning into less sensitive tissues like apoplast or vacuole thereby leading to mitigation of oxidative damage (Syeed *et al.*, 2011). SA mediates prevention of excess accumulation of Na in shoot tissues and protects the photosynthetic apparatus from any possible damage (Syeed *et al.*, 2011).

Low Na^+ concentration in shoots together with the reduced appearance of symptoms of foliage problems can be considered as relevant parameters for characterizing salt resistance as reflected from the results of present study (Eker *et al.*, 2006). (Saqib *et al.*, 2005) has observed in wheat, accumulation of low Na^+ concentrations in the shoot contributed substantially to increase the fresh and dry weights of salt tolerant cultivars. In the present study, exogenous SA application significantly contributed to reduce

Na^+ concentrations in the leaves to minimum possible for exploiting its beneficial role. Such positive influence of SA was obvious in reduced number of necrotic leaves possibly through reduced uptake of Na^+ (Fig. 4 and Table 1). Higher salinity treatment (125 mM NaCl) resulted in the development of specific necrotic symptoms on the old foliage. SA application at 0.5 mM protected leaves from the salinity induced necrosis and also resulted in development of healthier leaves under controlled conditions compared to control plants. However, at 0.75 mM SA, significant increment in the number of necrotic leaves was observed suggesting the concentrations to be toxic for wheat cultivar. Furthermore, salt stress, either alone or combined with high SA levels, increased the number of necrotic leaves by more than that of the non-saline-treated control particularly at 0.75 mM SA + 125 mM NaCl. Therefore, from the present investigation it could be inferred that lower Na^+ concentration in the shoots protect leaf development and impart salt tolerance in tissues as seen as reduced appearance of necrotic leaf spots in SA (0.5 mM) supplemented plants.

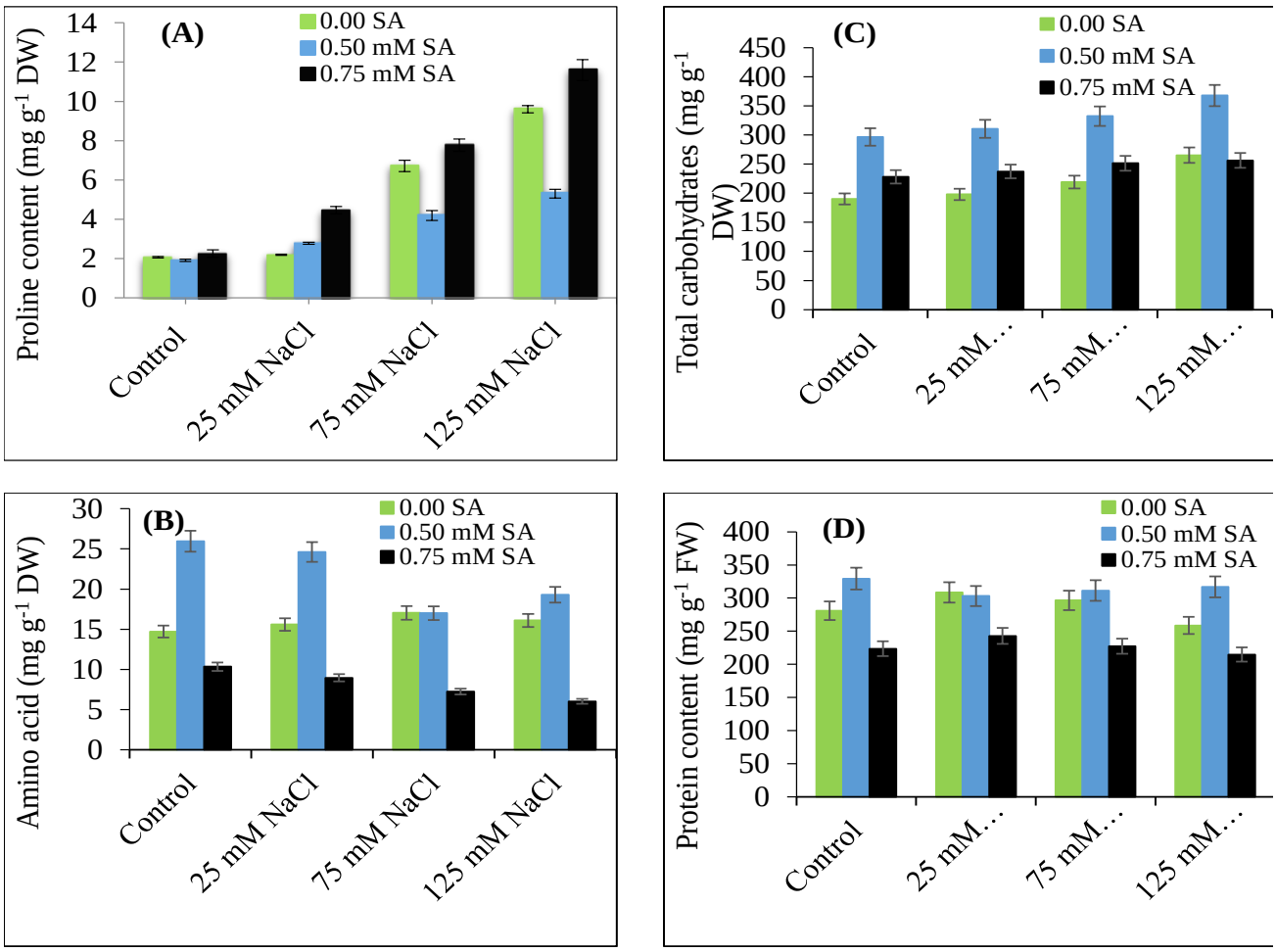

Fig. 3. Interactive effect of salicylic acid and different levels of NaCl on (A) proline (B) amino acids (C) total carbohydrates and (D) total protein of wheat plants. Values are means ± SD of three independent replications (n = 5).

Table 1. Interactive effect of salicylic acid and different levels of NaCl on Na^+ and K^+ ions concentration in shoot and root of wheat 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 $p \leq 0.05$

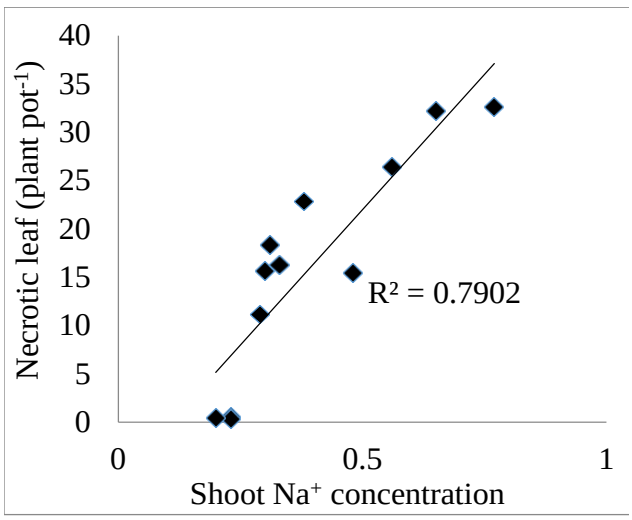

Fig. 4. Interactive effect of salicylic acid and different levels of NaCl on correlation between shoot Na+ concentration and necrotic leaf per plant of wheat plants. Values are means ± SD of three independent replications (n = 5).

Antioxidant-enzyme activities: The activity of SOD, CAT and APX in relation to NaCl and SA supplementation are presented in Fig. 5A-C. SOD activity enhanced by 5.27 fold, CAT activity by 3.28 fold and APX activity by 7.63 fold in 125 mM NaCl treated plants as compared to control. Supplementation of SA further enhanced the SOD activity by 1.31 fold with 0.50 mM SA and 1.76 fold with 0.75 mM SA as compared to NaCl alone treated plants.

CAT and APX activity was further enhanced by 1.77 fold and 2.12 fold with 0.50 mM NaCl respectively as compared to NaCl alone treated plants. SA (0.75 mM) increased CAT activity by 2.51 fold and APX activity by 2.22 fold with respect to NaCl alone treated plants (Fig. 5C-D).

Increased antioxidant activity has been reported to impart protection to numerous plant species, such as *Azolla* (Masood *et al.*, 2012), *Medicago truncatula* (Mhadhbi *et al.*, 2011), broad bean and wheat (Mohamed & Shaddad, 2013). Alscher *et al.,* (2002) reported that increased ROS scavenging through increased activities of antioxidant enzymes improved salt tolerance in wheat. SA application improved salt stress tolerance through enhanced activities of antioxidant enzymes in the current study. These results corroborate with the findings of Ahmad *et al.,* (2011) in *Brassica juncea* and Guo *et al.,* (2007) in *Oryza sativa*. SA has been reported to induce activity of antioxidant enzymes under different stresses like, drought stress in zoysiagrass (Chen *et al.*, 2014), chilling stress in maize (Janda *et al.*, 1999), heavy metal stress in rice (Mishra & Choudhuri, 1999). SA induced enhancement in the activity of CAT and APX in wheat plants contributed to quick neutralization of H_2O_2 and hence protecting the membranes from the toxic effects of stress. SA mediated increase in CAT activity under salt stress has also been reported by Gengmao *et al.,* (2014).

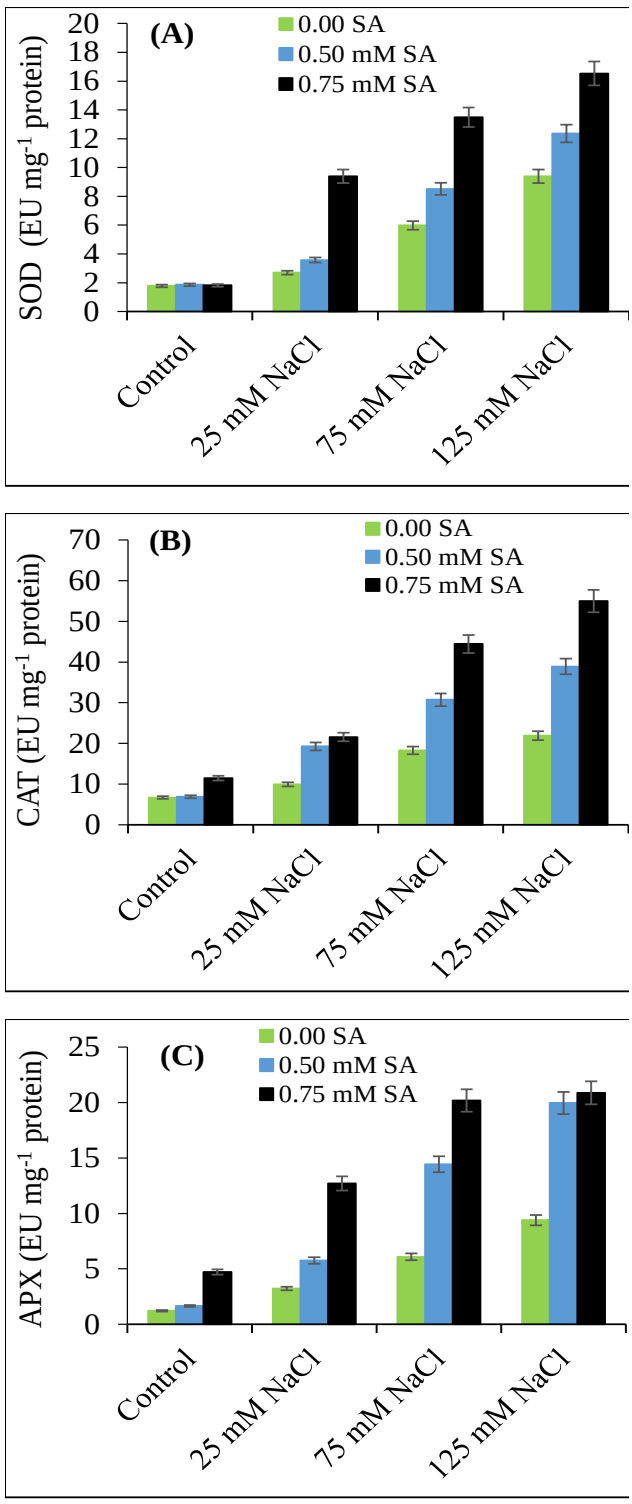

Fig 5. Interactive effect of salicylic acid and different levels of NaCl on activity of (A) SOD (B) CAT and (C) APX in wheat plants. Values are means ± SD of three independent replications (n = 5).

Conclusion

This study illustrates that NaCl stress has negative impact on the growth and physio-biochemical attributes of wheat seedlings. However, supplementation of salicylic acid improved the growth and development through the modulation of osmo-protectants and

enhanced the activity of enzymatic antioxidant system (SOD, CAT and APX). SA also reduced the accumulation of H_2O_2, MDA and lowered the permeability of the membrane. SA also hampers the uptake of shoot and root Na+ ions and increases the K+ uptake. Therefore, it is quite evident that application of appropriate SA concentration can be considered as effective stress ameliorating strategy in wheat seedlings.

Acknowledgements

This study was supported by the Deanship of Scientific Research and College of Science Research Center under research grant RG-1438-036, King Saud University, Saudi Arabia.

References

Aebi, H., 1984. *Catalase In vitro*. In: Methods Enzymol., S. Colowick and N. Kaplan, (Eds.). Elsevier, Florida: pp: 121-126.

Agamy, R., E. Hafez and T.H. Taha. 2013. Acquired resistant motivated by salicylic acid applications on salt stressed tomato (*Lycopersicon esculentum* Mill.). *The Amer.-Eura. J. Agri. & Environ. Sci.*, 13: 50-57.

Aggarwal, A., N. Kadian, K. Karishma, N. Neetu, A. Tanwar and K.K. Gupta. 2012. Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *J. Appl. & Nat. Sci.*, 4(1): 144-155.

Ahanger, M.A. and R. Agarwal. 2017a. Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L.). *Protoplasma*, 254(4): 1471-1486.

Ahanger, M.A. and R. Agarwal. 2017b. Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. *Plant Physiol. & Biochem.*, 115: 449-460.

Ahanger, M.A., M. Tittal, R.A. Mir and R. Agarwal. 2017. Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L., cultivars by potassium. *Protoplasma*, 254(5): 1953-1963.

Ahmad, P., A. Hashem, E.F. Abd-Allah, A.A. Alqarawi, R. John, D. Egamberdieva and S. Gucel. 2015. Role of *Trichoderma harzianum* in mitigating nacl stress in indian mustard (*Brassica juncea* L.) through antioxidative defense system. *Front. in Plant Sci.*, 6: 868.

Ahmad, P., A.A. Abdel Latef, A. Hashem, E.F. Abd-Allah, S. Gucel and L.S.P. Tran. 2016. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front. in Plant Sci.*, 7: 347.

Ahmad, P., C.A. Jaleel and S. Sharma. 2010. Antioxidant defense system, lipid peroxidation, proline-metabolizing enzymes, and biochemical activities in two *Morus alba* genotypes subjected to nacl stress. *Rus. J. Plant Physiol.*, 57(4): 509-517.

Ahmad, P., G. Nabi and M. Ashraf. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *S. Afri. J. Bot.*, 77(1): 36-44.

Ahmad, P., M. Ozturk, S. Sharma and S. Gucel. 2014. Effect of sodium carbonate-induced salinity–alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. *J. Plant Interact.*, 9(1): 460-467.

Ahmad, P., M.A. Ahanger, D. Egamberdieva, P. Alam, M.N. Alyemeni and M. Ashraf. 2018a. Modification of osmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea seedlings under mercury (hg) toxicity. *J. Plant Gro. Regul.*, 37(1): 309-322.

Ahmad, P., M.A. Ahanger, M.N. Alyemeni, L. Wijaya, D. Egamberdieva, R. Bhardwaj and M. Ashraf. 2017. Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) Czern & Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. *J. Plant Interact.*, 12(1): 429-437.

Ahmad, P., M.N. Alyemeni, M.A. Ahanger, D. Egamberdieva, L. Wijaya and P. Alam. 2018b. Salicylic acid (SA) induced alterations in growth, biochemical attributes and antioxidant enzyme activity in faba bean (*Vicia faba* L.) seedlings under nacl toxicity. *Rus. J. Plant Physiol.*, 65(1): 104-114.

Alamgir, A.N.M., M. Musa and M.Y. Ali. 2008. Some aspects of mechanisms of nacl stress tolerance in the seedlings of four rice genotypes. *Bang. J. Bot.*, 36(2): 181-184.

Alscher, R.G., N. Erturk and L.S. Heath. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.*, 53(372): 1331-1341.

Alzahrani, S.M., I.A. Alaraidh, H. Migdadi, S. Alghamdi, M.A. Khan and P. Ahmad. 2019. Physiological, Biochemical and antioxidant properties of two genotypes of Vicia faba grown under salinity stress. *Pak. J. Bot.*, 51(3): 786-798.

Ashraf, M. and M. Foolad. 2007. Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. *Environ. & Exp. Bot.*, 59: 206-216.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 166(1): 3-16.

Azooz, M.M., A.M. Youssef and P. Ahmad. 2011. Evaluation of salicylic acid (sa) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. *Int. J. Plant Physiol. & Biochem.*, 3(14): 253-264.

Bali, S., P. Kaur, A. Sharma, P. Ohri, R. Bhardwaj, M. Alyemeni, L. Wijaya and P. Ahmad. 2018. Jasmonic acid-induced tolerance to root-knot nematodes in tomato plants through altered photosynthetic and antioxidative defense mechanisms. *Protoplasma*, 255(2): 471-484.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant & Soil*, 39(1): 205-207.

Chen, Z.L., X.M. Li and L.H. Zhang. 2014. Effect of salicylic acid pretreatment on drought stress responses of zoysiagrass (*Zoysia japonica*). *Rus. J. Plant Physiol.*, 61(5): 619-625.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.,* 135: 1-9.

Eker, S., G. Cömertpay, Ö. Konuşkan, A.C. Ülger, L. Öztürk and İ. Çakmak. 2006. Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties. *Turk. J. Agri. & Fores.*, 30(5): 365-373.

Eyidogan, F. and M.T. Öz. 2007. Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiologiae Plantarum*, 29(5): 485-493.

Fales, F.W. 1951. The assimilation and degradation of carbohydrates by yeast cells. *J. Biol. Chem.*, 193(1): 113-124.

Gengmao, Z., S. Quanmei, H. Yu, L. Shihui and W. Changhai. 2014. The physiological and biochemical responses of a medicinal plant (*Salvia miltiorrhiza* L.) to stress caused by various concentrations of NaCl. *PLos One*, 9(2): e89624.

Ghassemi, F., A.J. Jakeman and H.A. Nix. 1995. *Salinisation of land and water resources: Human causes, extent, management and case studies.* CAB international, ISBN: 0851989063, 9780851989068.

Giannakoula, A.E. and I. Ilias. 2013. The effect of water stress and salinity on growth and physiology of tomato (*Lycopersicon esculentum* Mil.). *Arch. of Biol. Sci.*, 65(2): 611-620.

Gunes, A., A. Inal, M. Alpaslan, F. Eraslan, E.G. Bagci and N. Cicek. 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.*, 164(6): 728-736.

Guo, B., Y.C. Liang, Y.G. Zhu and F.J. Zhao. 2007. Role of salicylic acid in alleviating oxidative damage in rice roots (*Oryza sativa*) subjected to cadmium stress. *Environm. Pollut.*, 147(3): 743-749.

Hajihashemi, S., K. Kiarostami, S. Enteshari and A. Saboora. 2006. The effects of salt stress and paclobutrazol on some physiological parameters of two salt-tolerant and salt-sensitive cultivars of wheat. *Pak. J. Biol. Sci.*, 9(7): 1370-1374.

Hartzendorf, T. and H. Rolletschek. 2001. Effects of NaCl-salinity on amino acid and carbohydrate contents of *phragmites australis*. *Aquat. Bot.*, 69(2-4): 195-208.

Hasanuzzaman, M., K. Nahar, A. Rahman, T.I. Anee, M.U. Alam, T.F. Bhuiyan, H. Oku and M. Fujita. 2017. *Approaches to enhance salt stress tolerance in wheat*. In: *Wheat Improvement, Management and Utilization*. (Eds.): Wanyera, R. and J. Owuoche. Intech Open, London: pp: 151-187.

Heidari, M. and A. Golpayegani. 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J. Saudi Soci. Agri. Sci.*, 11(1): 57-61.

Iqbal, M., I. Saliha, M. Nadeem, F. Tatheer and A.B. Itrat. 2018. Salinity effects on wheat (*Triticum aestivum* L.) characteristics. *Int. J. Biosci.*, 12(3): 131- 146.

Iqbal, M., M. Ashraf, A. Jamil and S. ur-Rehman. 2006. Does seed priming induce changes in the levels of some endogenous plant hormones in hexaploid wheat plants under salt stress? *J. Integr. Plant Biol.*, 48(2): 181-189.

Jaleel, C.A., B. Sankar, R. Sridharan and R. Panneerselvam. 2008. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turk. J. Biol.*, 32(2): 79-83.

Janda, T., G. Szalai, I. Tari and E. Páldi. 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. *Planta*, 208(2): 175-180.

Jogaiah, S., S.R. Govind and L.-S.P. Tran. 2013. Systems biology-based approaches toward understanding drought tolerance in food crops. *Crit. Rev. Biotechnol.*, 33(1): 23-39.

Khan, M.I.R., N. Iqbal, A. Masood, T.S. Per and N.A. Khan. 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Sign. & Beh.*, 8(11): e26374.

Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. *Mod. Appl. Sci.*, 3(3): 171-176.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193(1): 265-275.

Ma, X., J. Zheng, X. Zhang, Q. Hu and R. Qian. 2017. Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyllaceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. *Front. Plant Sci.*, 8: 600.

Masood, S., L. Saleh, K. Witzel, C. Plieth and K.H. Mühling. 2012. Determination of oxidative stress in wheat leaves as influenced by boron toxicity and nacl stress. *Plant Physiol. & Biochem.*, 56: 56-61.

Mhadhbi, H., V. Fotopoulos, P.V. Mylona, M. Jebara, M. Elarbi Aouani and A.N. Polidoros. 2011. Antioxidant gene-enzyme responses in *Medicago truncatula* genotypes with different degree of sensitivity to salinity. *Physiologia Plantarum*, 141(3): 201-214.

Mir, M.A., G. Sirhindi, M.N. Alyemeni, P. Alam and P. Ahmad. 2018. Jasmonic acid improves growth performance of soybean under nickel toxicity by regulating nickel uptake, redox balance, and oxidative stress metabolism. *J. Plant Gro. Regul.,* 37(4): 1195-1209.

Mishra, A. and M.A. Choudhuri, 1999. Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant.*, 42(3): 409-415.

Misra, N. and P. Saxena. 2009. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.*, 177(3): 181-189.

Mohamed, A.S.H. and M.A.K. Shaddad. 2013. Effect of salinity on dry matter production, ion accumulation, some metabolites and antioxidant enzymes in wheat and broad bean. *Al-Azhar Bulet. of Sci.*, 24(2): 149-164.

Moharekar, S.T., S.D. Lokhande, T. Hara, R. Tanaka, A. Tanaka and P.D. Chavan. 2003. Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. *Photosynthetica*, 41(2): 315-317.

Moore, S. and W.H. Stein. 1948. Photometric nin-hydrin method for use in the chromatography of amino acids. *J. Biol. Chem.*, 176: 367-388.

Mukherjee, S. and M. Choudhuri. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plantarum*, 58(2): 166-170.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. in Plant Biol.*, 59(1): 651-681.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant & Cell Physiol.*, 22(5): 867-880.

Naureen, G. and F. Naqvi. 2010. Salt tolerance classification in wheat genotypes using reducing sugar accumulation and growth characteristics. *Emi. J. Food & Agri.*, 22(4): 308.

Oliver, M.J., P. O'mahony and A.J. Wood. 1998. "To dryness and beyond"–preparation for the dried state and rehydration in vegetative desiccation-tolerant plants. *Plant Growth Regul.*, 24(3): 193-201.

Orsini, F., M. Alnayef, S. Bona, A. Maggio and G. Gianquinto. 2012. Low stomatal density and reduced transpiration facilitate strawberry adaptation to salinity. *Environ. & Exp. Bot.*, 81: 1-10.

Palma, F., M. López-Gómez, N.A. Tejera and C. Lluch. 2013. Salicylic acid improves the salinity tolerance of *Medicago sativa* in symbiosis with *Sinorhizobium meliloti* by preventing nitrogen fixation inhibition. *Plant Sci.,* 208: 75-82.

Pitann, B., A. Mohamed, A.B. Neubert and S. Schubert. 2013. Tonoplast Na^+/H^+antiporters of newly developed maize (*Zea mays*) hybrids contribute to salt resistance during the second phase of salt stress. *J. Plant Nutr. & Soil Sci.*, 176(2): 148-156.

Rao, M.K.V. and T.V.S. Sresty. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) millspaugh) in response to Zn and Ni stresses. *Plant Sci.,* 157(1): 113-128.

Rasool, S., A. Ahmad, T.O. Siddiqi and P. Ahmad. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol. Plantarum,* 35(4): 1039-1050.

Saleem, A., M. Ashraf and N.A. Akram. 2011. Salt (nacl)-induced modulation in some key physio-biochemical attributes in okra (*Abelmoschus esculentus* L.). *J. Agron. & Crop. Sci.*, 197(3): 202-213.

Saqib, M., J. Akhtar and R.H. Qureshi. 2005. Na^+ exclusion and salt resistance of wheat (*Triticum aestivum*) in saline-waterlogged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. *Plant Sci.*, 169(1): 125-130.

Schlegel, H.G. 1956. Die verwertung organischer säuren durch chlorella im licht. *Planta*, 47(5): 510-526.

Schubert, S., A. Neubert, A. Schierholt, A. Sümer and C. Zörb. 2009. Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods. *Plant Sci.*, 177(3): 196-202.

Senadheera, P., S. Tirimanne and F.J.M. Maathuis. 2012. Long term salinity stress reveals variety specific differences in root oxidative stress response. *Rice Sci.*, 19(1): 36-43.

Shaddad, M.A., A.F. Radi, A.M. Abdel-Rahman and M.M. Azooz. 1990. Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. *Plant & Soil*, 122(2): 177-183.

Shen, C., Y. Hu, X. Du, T. Li, H. Tang and J. Wu. 2014. Salicylic acid induces physiological and biochemical changes in *Torreya grandis* cv. Merrillii seedlings under drought stress. *Trees*, 28(4): 961-970.

Siringam, K., N. Juntawong, S. Cha-um, T. Boriboonkaset and C. Kirdmanee. 2012. Salt tolerance enhancement in indica rice ('*Oryza sativa*' L. spp. Indica) seedlings using exogenous sucrose supplementation. *Plant Omics*, 5(1): 52.

Syeed, S., N.A. Anjum, R. Nazar, N. Iqbal, A. Masood and N.A. Khan. 2011. Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. *Acta Physiologiae Plantarum*, 33(3): 877-886.

Türkan, I. and T. Demiral. 2009. Recent developments in understanding salinity tolerance. *Environ. & Exp. Bot.*, 67(1): 2-9.

van Rossum, M.W., M. Alberda and L.H. van der Plas. 1997. Role of oxidative damage in tulip bulb scale micropropagation. *Plant Sci.*, 130(2): 207-216.

Yin, Y.-G., Y. Kobayashi, A. Sanuki, S. Kondo, N. Fukuda, H. Ezura, S. Sugaya and C. Matsukura. 2010. Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (*Solanum lycopersicum* L. Cv. 'Micro-tom') fruits in an ABA- and osmotic stress-independent manner. *J. Exp. Bot.*, 61(2): 563-574.

Yusuf, M., S.A. Hasan, B. Ali, S. Hayat, Q. Fariduddin and A. Ahmad. 2008. Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. *J. Integr. Plant Biol.*, 50(9): 1096-1102.

Zhang, L., H. Ma, T. Chen, J. Pen, S. Yu and X. Zhao. 2014. Morphological and physiological responses of cotton (*Gossypium hirsutum* L.) plants to salinity. *Plos One*, 9(11): e112807.

(Received for publication 10 July 2019)