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Full Length Research Paper

Alleviation of salinity effects by exogenous applications of salicylic acid in pearl millet (*Pennisetum glaucum* (L.) R. Br.) seedlings

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Experiments for the study of alleviation of salt stress effects by exogenous applications of salicylic acid (SA) in pearl millet (*Pennisetum glaucum* (L.) R. Br.) seedlings were conducted in soil filled earthen pots having size 12 inches with 7 kg soil. Three treatments comprising, control (T_0), 50-mol m^{-3} NaCl (T_1) and T_2 are having NaCl (50 mol m^{-3}) + SA (50 mg l^{-1}). Pots were arranged in completely randomized design (CRD) with 6 replicates. NaCl **significantly** reduced the **plant and root lengths, plant fresh and dry weights**. In contrast, NaCl did not show any adverse effect on plants treated with NaCl plus **SA. Salicylic acid treated pearl millet plants under NaCl salinity strongly reduced accumulations of Na^+, K^+, Ca^{2+} and Cl^- and glycinebetaine (GB) and total soluble carbohydrates (TSC) as compared to NaCl treatments. Higher N and relative water contents (RWC) was noted in T_2 (NaCl + SA) but it reduced in T_1 (NaCl) as compared to control. It was concluded that SA could be used as a potential growth regulator to improve salt tolerance in plants.**

Key words: Exogenous, salicylic acid, growth, ion contents, salt tolerance, pearl millet.

INTRODUCTION

Many species of higher plants, including most crops, are subjected to growth inhibition under high NaCl conditions. The salt-induced inhibition of plant growth is caused not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism. While the first component can bring about water deficit, the excess of a specific ion can cause toxicity and can induce nutritional disorders (Khatoon et al. 2010).

Salinity is the process of accumulation of soluble slats, by which saline soils are produced. The composition of salts in large amounts mostly are calcium, sodium, magnesium, chloride and sulphate ions and in relatively small amounts are potassium, carbonates, bicarbonates, borate and lithium salts (Zhu, 2001). Accumulation of these salts increases the osmotic pressure of the soil solution because of restricted water intake by plants (Cramer et al. 1999).

Several reports appearing in the literature revealed that salinity causes many adverse effects on the morphology, anatomy and physiology of pearl millet (Hussain et al., 2010). For instance, percent germination, height, grain and straw yield of pearl millet decreased with increasing concentration of salinity (Hussain et al., 2008).

When plants are exposed to salt stress, they adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa et al., 2000). Salicylic acid (SA) plays an

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Abbreviations: SA, Salicylic acid; **GB,** glycine betaine; **RWC,** relative water contents; **TSC,** total soluble carbohydrates.

important role in the defense response to pathogen attack and stresses, like heat and water stresses in plant species (Shakirova et al. 2003).

Salicylic acid is water-soluble antioxidant compound that can also regulate plant growth. It also has a role in abiotic stress tolerance such as drought tolerance in wheat (Singh and Usha, 2003; Sakhabutdinova et al. 2003). Ameliorative results of salicylic acid on growth of crop plants under abiotic stress conditions may have been due to its role in nutrient uptake (Noreen and Ashraf, 2008).

Several studies also supported a major role of salicylic acid in modulating the plant response to several abiotic stresses including salt and water stress (Yalpani et al., 1994; Senaratna et al., 2000). Treating mustard seedlings with salicylic acid improved their thermotolerance and heat acclimation (Dat et al. 1998). In maize plants, pre-treatment with salicylic acid induced the production of antioxidant enzymes, which in turn increases chilling and salt tolerance (Janda et al., 1999).

The objective of the present study was to evaluate the effect of salinity on pearl millet and to assess the role of salicylic acid (SA) applications under salt stress by studying morphology, biochemical and physiological studies. Comparative study will be made for NaCl with NaCl + SA application.

MATERIALS AND METHODS

Seeds of pearl millet (Pennisetum glaucum (L.) R. Br.) line 18-By (salt sensitive) were obtained from the Maize and Millet Research Station, Yousafwala, District Sahiwal, Pakistan. Seeds were surface sterilized by dipping in 10% sodium hypochlorite solution for 10 min, then rinsed with sterilized distilled water (5-times) and air-dried at an ambient temperature of 32°C in the laboratory. Following treatments of NaCl and SA were applied after 21days of seeds germination.

T0 = Control
T1= 50 mol m–3 NaCl
T2= 50 mol m–3 NaCl + SA (50 mg l-1)

NaCl was applied in soil media and SA was applied as foliar spray after 14 days of germination. There were total 30 pots comprising 10 pots for each treatment. Experiments were laid out in Completely Randomized Design (CRD) with six replicates. Plants were harvested after 120 days of treatment and following studies were made during both years of experiments.

Growth attributes and ion contents

Plants were uprooted carefully and washed in distilled water. Plant and root length was measured. Shoot fresh weight (g) was recorded by electronic balance. Plant samples were placed in an oven at 75°C for 7 days. After 7 days shoot and root dry weight (g) was recorded again.

Dried plant material was finely ground and digested with a nitric-perchloric mixture. In leaves and roots ion contents of Na^+ and K^+ were determined by emission spectrophotometry and Ca^{2+} by atomic absorption spectrophotometry (Allan, 1969). Total nitrogen was estimated by Kjeldhal procedure (Bremner, 1965). Chloride was extracted by stirring ground-dried samples with 0.1 M $NaNO_3$ for 30 min. After extract clarification with activated coal, it 13.2 mM $Hg(SCN)_2$ was added in methanol and 20.2% (w/v) $Fe(NO_3)_3$ (4 + 1) and absorbance was determined at 460 nm (Gaines et al. 1984).

Total soluble carbohydrates (µg g^{-1} DW)

Total soluble carbohydrates (TSC) concentrations were determined according to method of Cahi and Brun (1978). Samples of 100 mg of roots and leaves were homogenized with 10 ml of extracting solution (glacial acetic acid: methanol: water, 1:4:5, v/v/v). The homogenate was centrifuged for 10 min at 3,000 rpm and the supernatant was decanted. The residue was resuspended in 10 ml of extracting solution and centrifuged another 5 min at 3,000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 ml with water. For measurement of TSC, a phenol-sulfuric acid assay was used as described by Dubois et al. (1956). A volume of 0.5 ml of 5% (v/v) phenol solution and 2.5 ml of concentrated sulfuric acid were added to 0.5 ml aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometry at 490 nm (Shimadzu spectrophotometer, Duisburg, Germany).

Leaf relative water contents (%)

The leaf relative water contents (RWC) were calculated at the time harvest according to Beadle et al. (1993) using the equation:

RWC (%) = [(FW - DW)/ (TW - DW)] 100

Where FW is fresh weight, DW is dry weight, and TW is turgid weight.

Glycinebetaine and proline (µg g^{-1} DW)

Glycinebetaine was extracted by stirring finely ground-dried samples of roots with demineralized water at 100°C for 1 h. Glycinebetaine contents were determined spectrophotometrically after reaction with KI-I_2 at 365 nm (Grieve and Grattan, 1983). Proline was also determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973) using L-proline as a standard. Approximately 300 mg of dry tissue was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and filtered. In 2 ml of the filtrate, 2 ml of acid ninhydrin was added, followed by the addition of 2 ml of glacial acetic acid and boiled for 60 min. The mixture was extracted with toluene and the free proline was quantified spectro-photometrically at 520 nm from the organic phase using a Shimadzu spectrophotometer (Duisburg, Germany).

Isolation of salicylic acid (SA)

Salicylic acid was measured according to the method of Meuwly and Métraux (1993).

Statistical analysis

Analysis of variance (ANOVA) technique was employed for carrying out statistical analysis of data collected (Steel and Torie, 1980). The means values were compared with Least Significant Difference (LSD) Test, following Snedecor and Cochran (1980).

Table 1. Comparison of means of exogenous salicylic acid applications for salt tolerance in pearl millet.

Attribute	T_0 (0 mol m^{-3})	T_1 (50 mol m^{-3} NaCl)	T_2 (50 mol m^{-3} NaCl + SA)	LSD at 5%
Plant length (cm)	149.9 ± 2.4 a	125.4 ± 1.6 b	147.1 ± 3.6 a	4.8
Root length (cm)	18.1 ± 1.1 a	16.9 + 1.1 b	18.4 ± 1.4 a	2.9
Plant fresh weight (g)	31.0 + 1.7a	38.1 ± 0.07 b	30.8 ± 1.9 a	1.6
Plant dry weight (g)	16.2 ± 1.2 a	13.9 ± 0.09 b	16.1 ± 0.09 a	1.4
Na^+ (ppm) in roots	17.2 ± 1.4 b	26.6 + 1.8 a	16.8 + 1.5 b	3.5
Na^+ (ppm) in leaves	34.5 ± 3.2 b	72.2 + 1.2 a	32.1 ± 2.5 b	3.1
K^+(ppm) in roots	866.2 + 4.3 b	1033.2 ± 2.9a	845.2 ± 2.1 b	15.9
K^+(ppm) in leaves	621.1 ± 3.3 b	721.6 ± 2.4 a	632.0 ± 2.5 b	21.5
Ca^{2+} (ppm) in roots	140.1 + 6.5 a	50.0 ± 1.12 b	137.2 ± 1.6 a	10.2
Ca^{2+} (ppm) in leaves	39.7 ± 5.2 a	32.6 + 1.9 b	41.2 + 2.2 a	3.6
Cl^- (ppm) in roots	140.3 ± 4.9 b	276.7 ± 1.3 a	136.5 ± 1.6 b	10.9
Cl^- (ppm) in leaves	209.2 ± 2.1 b	328.2 + 1.6 a	202.9 ± 1.4 b	18.7
N (%) in roots	1.6 + 0.6 b	1.1 ± 0.7 c	2.1 ± 0.1 a	0.21
N (%) in leaves	2.4 + 1.02 b	1.2 + 0.4 c	2.9 ± 0.2a	0.32
TSC (µg g^{-1} DW) in roots	745 ± 4.6 b	970 ± 3.4 a	743 ± 5.6 b	6.6
TSC (µg g^{-1} DW) in roots	633 ± 2.3 b	720 ± 4.1 a	621 ± 4.4 b	5.3

Small letter indicates statistical difference among different treatments (values with +, ± are standard deviations). LSD, Least significant difference; T_0, control group; $T_1 – T_2$, treatment groups.

RESULTS AND DISCUSSION

Growth attributes

Comparison of treatments means for growth attributes as plant and root lengths, plant fresh and dry weights showed that T_1 (50 mol m^{-3} NaCl) applications reduced plant and root lengths, plant fresh and dry weights over control. These, however, increased when salicyclic acid was applied; differences between T_o and T_2 were non significant (Table 1). It also showed that salicyclic acid assisted the pearl millet plants to eradicate the effect of NaCl stress.

Ion contents

Impact of NaCl stress was highly significant for ions accumulations in pearl millet plants. Na^+, K^+ and Cl^- concentrations were higher in T_1 (50 mol m^{-3} NaCl) over T_0 (control) both in roots and leaves. It was noted that concentrations of these ions were higher in leaves as compared to roots. In contrasts, salicyclic acid treated plants showed non-significant effect of salt for these ions in comparison with salt treated plants and control. N contents were reduced in T_1, while T_2 plants had higher N contents both in roots and leaves than control. Ca^{2+} concentrations were decreased by NaCl stress while in plants sprayed with salicyclic acid had non-significant effect on Ca^{2+} accumulation (Table 1).

Total soluble carbohydrates (µg g^{-1} DW)

Root and leaves total soluble carbohydrates (TSC) concentrations increased sharply in relation to the salt stress (T_1), while it had non-significant effect in T_2 treated with salicyclic acid under NaCl (Table 1). It probably reflected the maintenance or even induction of root elongation at low water potentials, which could be considered as an adaptive response to salinity.

Relative water contents (%)

Salt stress lowered the relative water contents (RWC) significantly under NaCl stress (T_1). It decreased below to 60% RWC. On the other hand treatment T_2 (SA + NaCl) had constant RWC as control that was above 75% (Figure 1). This reduction in RWC might be resulted in decline of plant growth attributes.

Glycine betaine (µg g^{-1} DW)

The osmotic adjustment would be accomplished by the accumulation of organic solutes. Among the organic solutes investigated, glycine betaine (GB) showed the highest absolute accumulation in response to salinity (T_1 treatment) that was above that 40-µg g^{-1} DW. In control, GB was below 25 µg g^{-1} DW. In contrast, treatment T_2 (SA + NaCl) showed significant reduction in GB

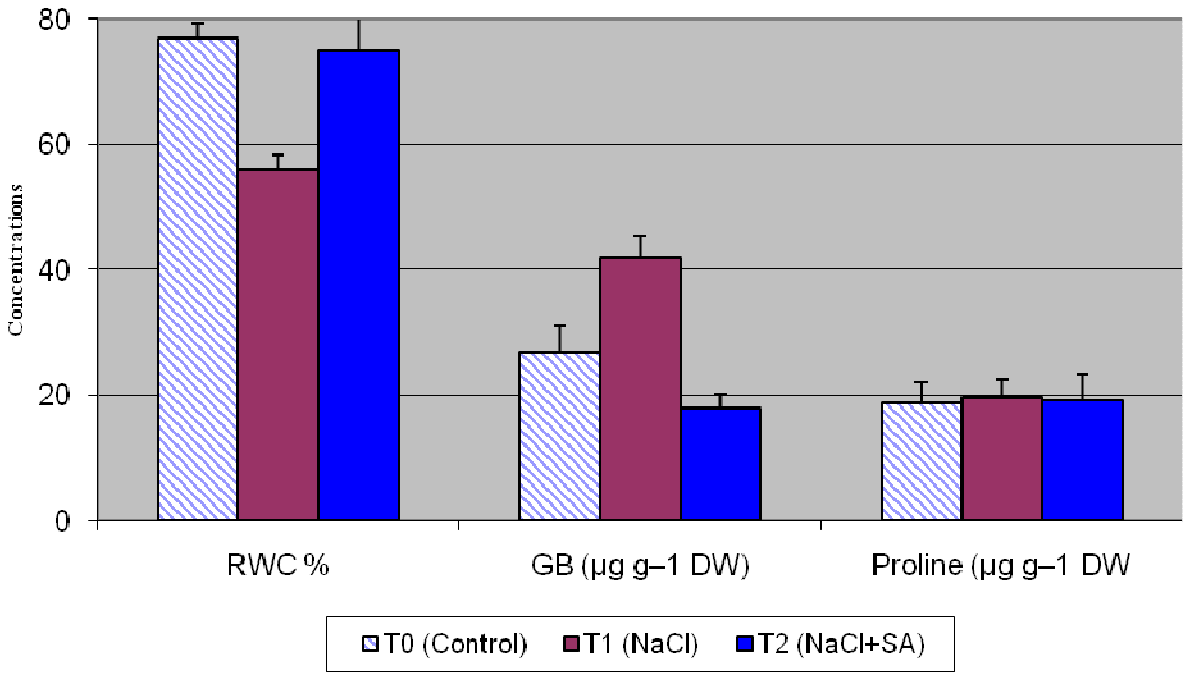

Figure 1. Effects of exogenous applications of SA in pearl millet seedlings for RWC (%), glycinebetaine and proline (µg g–1 DW) under NaCl.

accumulations that was below 20-µg g^{-1} DW (Figure 1).

Proline (µg g^{-1} DW)

Proline accumulation was not significantly affected by salinity (Figure 1). Proline concentrations were statistically equal in all treatments. Contrary to its generally accepted role in many other plant species, proline plays an important role in the mechanism of salt tolerance. The significance of proline accumulation in osmotic adjustment is still debated and varies according to the plant species.

Salicylic acid (µg g^{-1} FW)

It was noted that T_2 (SA + NaCl) had significantly higher accumulations of salicyclic acid (SA) as compared to control (Figure 2.). NaCl stress had non-significant effect on salicyclic acid accumulations in pearl millet (T_1) both in roots as well as leaves. Both control and T_1 had almost equal concentrations of salicyclic acid.

Salt (NaCl) stress is among the factors most limiting to plant productivity (Shi et al., 2002). Plants exposed to salt stress adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hussain et al., 2008). The reason for growth reduction in pearl millet could be due to water shortage and ionic toxicity caused by salinity. The increase in plant growth may be due to turgor potential which is decreased by water deficit produced by high concentrations of the salts in the soil (Haung and Redmann, 1995). Assessment of pattern of accumulation of toxic ions in different ions in different plant parts is of vital importance to understand as to whether salt resistant or sensitive in toxic ions present in its growth medium. It also affects the enzyme activities of plants. Pearl millet plants under NaCl salinity showed accumulations of Na^+, K^+, Ca^{2+} and Cl^- ions and changes in enzyme activities. Similar results of ion accumulations and enzymes activities have been earlier found in Atriplex by Khan, (2000). Similar results of accumulations of inorganic ions in salt sensitive and resistant pearl millet lines were described by Hussain et al. (2008). These results are also in accordance with Hussain et al. (2009) and Meloni et al. (2001). Similar results for GB and proline under NaCl were found by many scientists in tomato (Heuer, 2003) and in rice (Lutts et al., 1996). Salicyclic acid plays an important role in the defense response to stresses (salts, water, etc) in many plant species (Yalpani et al., 1994; Senaratna et al., 2000). Exogenously applications of salicyclic acid helped to increase plant growth significantly in saline conditions (Setevens et al., 2007). Exogenously applications of salicyclic acid strongly inhibited Na^+, K^+, Ca^{2+} and Cl^- and organic solute accumulations (GB and TSC) but stimulated N and RWC (Shirasu, 1997).

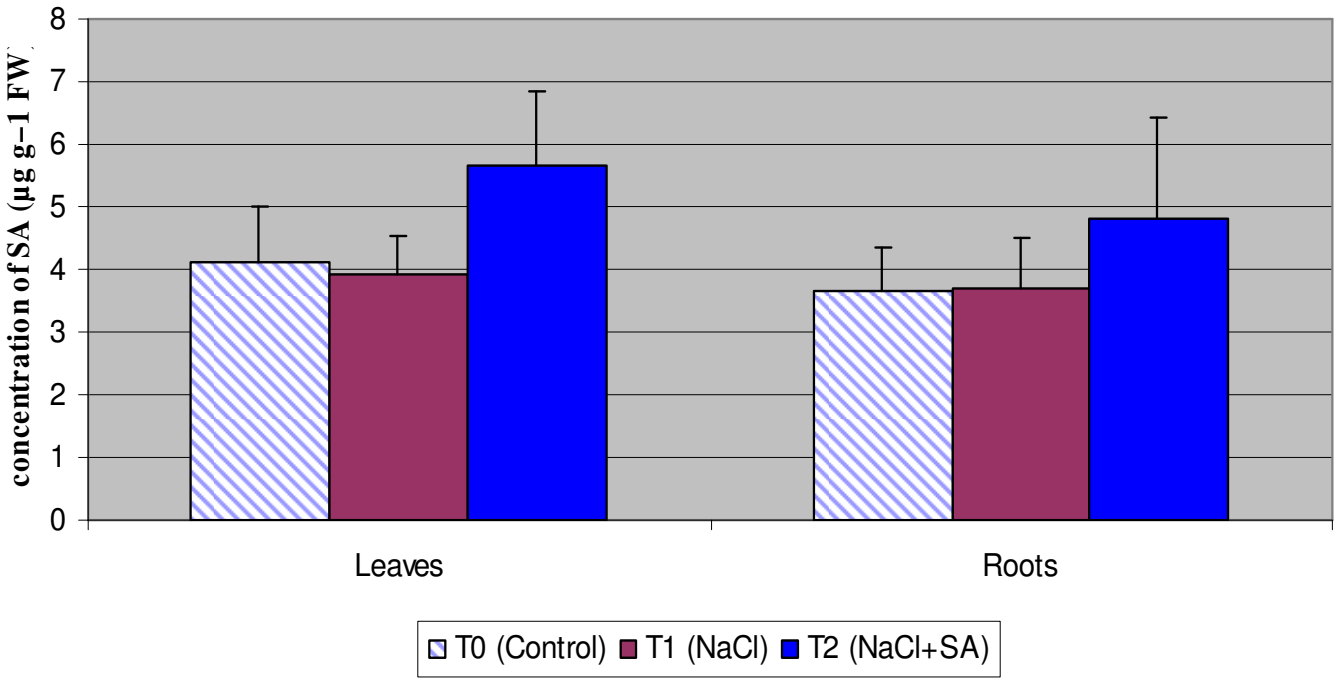

Figure 2. Effects of exogenous applications of SA in pearl millet seedlings under NaCl.

Conclusions

It is concluded that salicyclic acid may be used as a potential growth regulator to improve plant salinity stress tolerance. However, amount and timing of exogenous application has to be refined with further experimentation separately for each crop.

REFERENCES

Allan JE (1969). The preparation of agricultural samples for analysis by atomic absorption spectroscopy. Var. Techtron, p. 15.

Bates LS, Waldren RP, Tear ID (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.

Beadle CL, Ludlow MM, Honeysett L (1993). Water relations. In: Hall DO, Scurlock JMO, Bolhàr-Nordenkampf HR, Leegood RC, Long SP (eds). Photosynthesis and Production in a Changing Environment, Chapman and Hall, London, England. pp. 113-127.

Bremner JM (1965). Total nitrogen and inorganic forms of nitrogen. In methods of soil analysis. Ed. Black CA. Soc. Agron. Madison. Wisconsin: 2: 1149-1237.

Cahi K, Brun AJ (1978). Adaptation to environmental stresses. Plant Cell, 7: 1099-1111.

Cramer GR, Basset RA, Seemann JR (1999). Salinity calcium interaction on root growth and osmotic adjustment of two corn cultivars differening in salt tolerance. J. Plant Nutr. 13(11): 1453-1462.

Dat JF, Lopez DH, Foyer CH, Scott IM (1998). Parallel changes in H2O2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol. 116: 1351-1357.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Gaines TP, Parker MB, Gascho GJ (1984). Automated determination of chlorides in soil and plant tissue by sodium nitrate. Agron. J. 76: 371-374.

Grieve CM, Grattan SR (1983). Rapid assay for determination of watersoluble quaternary-amino compounds. Plant Soil, 70: 303-307.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51: 463-499.

Haung J, Redmann RE (1995). Responses of growth, morphology and anatomy to salinity and calcium supply in cultivated and wild barely. Can. J. Bot. 73: 1859-1866.

Heuer B (2003). Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. Plant Sci. 165: 693-699.

Hussain K, Ashraf M, Ashraf MY (2008). Relationship between growth and ion relation in pearl millet (*Pennisetum glaucum* (L.) R. Br.) at different growth stages under salt stress. Afr. J. Plant Sci. 2(3): 23-27.

Hussain K, Majeed A, Nawaz K, Bhatti KH, Nisar FK (2009). Effect of different levels of salinity on growth and ion contents of Black Seeds (*Nigella sativa* L.). Curr. Res. J. Biol. Sci. 1(3): 135-138.

Hussain K, Majeed A, Nawaz K, Nisar FK, Khan F, Afghan S and Ali K (2010). Comparative study for salt stress among seed, root stock and direct regenerated violet (*Viola odorata* L.) seedlings in relation to growth, ion contents and enzyme activities. Afr. J. Biotechnol. 9(14): 2108-2117.

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

Khan AA (2000). Preplant physiological seed conditioning, Hortic. Rev. 13: 131-181

Khatoon T, Hussain K, Majeed A, Nawaz K, Nisar MF (2010). Morphological variations in Maize (*Zea mays* L.) under different levels of NaCl at germinating stage. World Appl. Sci. J. 8(10): 1294-1297.

Lutts S, Kinet JM, Bouharmont J (1996). Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. J. Plant Growth Regul. 19: 207-218.

Meloni DA, Oliva MA, Ruiz HA, Martinez CA (2001). Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. J. Plant Nutr. 24: 599-612.

Meuwly P, Métraux JP (1993). Ortho-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. Anal. Biochem. 214: 500-505.

Noreen S, Ashraf M (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. Pak. J. Bot. 40(4): 1657-1663.

Sakhabutdinova A, Fatkutdinova DR, Bezrukova MV, Shakirova FM (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg. Special Issue, J. Plant. Physiol. 21: 314-319.

Senaratna T, Touchell D, Bunn T, Dixon K (2000). Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul. 30: 157-161.

Shakirova MF, Sakhabutdinova MV, Bezrukova RA, Fatkhutdinova FD (2003). Changes in the hormonal status of pearl millet seedlings induced by salicylic acid and salinity. Plant Sci. 164(3): 317-322.

Shi H, Quintero FJ, Pardo JM, Zhu JK (2002). The putative plasma membrane Na^+/H^+ antiporter SOS1 controls long distance Na+ transport in plants. Plant Cell, 14: 465-477.

Shirasu K, Nakajima H, Rajashekar K, Dixon RA, Lamb C (1997). Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signal in the activation of defense mechanisms. Plant Cell, 9: 261-270.

Singh B, Usha K (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul. 39: 137-141.

Snedecor GW, Cochran WB (1980). Statistical Methods, Ed. 8 Iowa State University, Iowa, USA.

Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics with Special Reference to Biological Sciences, McGraw Hill Book Co. Inc., Singapore.

Yalpani N, Enyedi AJ, León J, Raskin I (1994). Ultrapearl millet light and ozone stimulate accumulation of salicylic acid and pathogenesis related proteins and virus resistance in tobacco. Planta, 193: 373-376.

Zhu JK (2001). Plant salt tolerance. Trends Plant Sci. 6: 66-71.