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GROWTH MODULATION AND ION PARTITIONING IN SALT STRESSED SORGHUM (*SORGHUM BICOLOR* L.) BY EXOGENOUS SUPPLY OF SALICYLIC ACID

TAHIR MAHMOOD[1], NAEEM IQBAL[1 *], HAMMAD RAZA[1], MUHAMMAD QASIM[1] AND M. YASIN ASHRAF[2]

[1]*Plant Physiology Lab, Department of Botany, GC University, Faisalabad, Pakistan*
[2] *Stress Physiology Lab., Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan*

Abstract

An experiment was conducted to investigate the possible involvement of exogenous salicylic acid in salinity tolerance of two sorghum (*Sorghum bicolor* L.) lines, viz., PARI-S-4 and YSS-9. Three salinity levels i.e., 0, 60 and 120 mmol NaCl were developed at the time of seed sowing. Foliar spray of salicylic acid (0, 25 and 50 mg L^{-1}) was applied 14 days after germination. Increasing salinity treatments reduced the fresh and dry mass of both root and shoot. Plants treated with salicylic acid showed no recovery from salt induced reduction in biomass production. Root/shoot fresh and dry mass ratio, however, was not affected by salinity and salicylic acid treatments. Salicylic acid treated plants showed no recovery from excessive accumulation of Na^+ in their shoot/root, when under salt stress. Reduction in the accumulation of K^+ in salt treated plants not compensated by exogenous application of salicylic acid. Salt stressed plants treated with salicylic acid, however, showed higher shoot Ca^{2+} than untreated ones. Exogenous application of 25 ppm of salicylic acid proved to be more effective in reducing the salt induced perturbance in shoot Ca^{2+} as compared to salicylic acid level of 50 mg L^{-1}. Shoot and root Na^+/ K^+ ratio though increased with increase in salinity levels, which was not reversed by exogenous supply of salicylic acid.

Introduction

Soil salinity, the presence of excessive salts in the soil, is a very serious problem for agricultural productivity (Munns, 2002; 2005). Salinity affects 7% of the world's land surface and is one of the main limiting constraints to global agricultural productivity. It is estimated that 20% of the cultivated land of the world is affected by various degrees of salinity, which inhibits plant growth and yield. Soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many arid and semi-arid regions of the world. In salt affected areas, plant growth is severely affected by salinity through water deficit and salt specific damages (Qureshi *et al*., 2007). There are many reports indicating that high level of these salts may cause a reduction in growth of the plants, especially in plant biomass production (Iqbal *et al*., 2006; Sepehr & Mahlagha, 2006). The deleterious effects of salinity were suggested because of water stress, ion toxicities, ion imbalance or combination of all these factors (Ashraf *et al*., 2005).

Presence of excessive salts in the growth medium adversely affects plant growth and various biochemical and physiological processes. For instance, it is reported that leaf area is inhibited by salinity (Brugnoli & Lauteri, 1991). The salinity also decreases photosynthetic activity (Sakamoto *et al*., 1998) and inhibits growth (Hu & Schmidhalter, 1998). It has also been reported that salt stress limits plant productivity causing disturbance in various physiological and biochemical processes (Feri *et al*., 2000; Zhang *et al*., 2005).

*Correspondence author E-mail: naeemgc@yahoo.com

Plants are immobile organisms. They cannot tolerate adverse environmental conditions as mobile organisms. In order to combat environmental stresses, plants show certain alterations in their metabolic processes. One such alteration is the excessive accumulation of organic metabolites and growth regulators under stressful environment (Rhodes & Hanson, 1993). Exogenous application of these metabolites/growth regulators to improve stress tolerance of crop plants has got attention by researchers from last few years (Afzal *et al*., 2005; Iqbal *et al*., 2005; Iqbal & Ashraf, 2007).

Salicylic acid plays an important role in the defense response in many plant species to biotic stresses. It acts as a signal for the development of the systematic acquired resistance (Shirasu *et al*., 1997). It is also reported that salicylic acid plays an important role in determining the sensitivity of plants to various abiotic stresses (Dat *et al*., 1998; Bandurska and Stroiński, 2005), particularly at the seedling stage. It supports to modulate the plant response to several abiotic stresses (Yalpani *et al*., 1994; Senaratna *et al*., 2000; Borsani *et al*., 2001). There are many reports indicating that exogenous application of salicylic acid is very helpful in reducing the adverse effects of biotic and abiotic stresses. For example, salicylic acid can either inhibit germination as in maize (Guan & Scandalios, 1995) or enhance seed vigor as in wheat (Shakirova *et al*., 2003) and pea (Mccue *et al*., 2000). In *Arabidopsis*, salicylic acid has been reported to increase the oxidative damage generated by NaCl and osmotic stresses, thus causing seedling lethality under these conditions (Borsani *et al*., 2001). It was also observed that exogenous application of salicylic acid improved salt tolerance in dicotyledons (Stevens, 1986; Borsani *et al*., 2001). Salicylic acid increases the fresh and dry weight of shoot and root of the salt stressed maize (Khodary, 2004). Salicylic acid also induces tolerance to salinity in wheat plants (Hamid *et al.,* 2008). Keeping in view the adverse effects of salinity on plants and the importance of salicylic acid in stress tolerance of plants, the present study was conducted to find out the role of salicylic acid in salinity tolerance of sorghum. The objective of the study was to find out whether and how exogenous supply of salicylic acid can ameliorate the adverse effects of salinity on nutrient accumulation and biomass production in sorghum at seedling stage.

Materials and Methods

The present investigation provides important information regarding the response of sorghum lines to exogenous application of salicylic acid under salt stress conditions. The work was carried out in sand culture at Department of Botany, Govt. College University, Faisalabad, Pakistan. Two Sorghum lines, Pari-S-4 and YSS-9 were used for the present study. The seeds of both lines were obtained from National Agriculture Research Centre, Islamabad, Pakistan. The seeds were surface sterilized with 10% Sodium hypochlorite solution for 4 minutes and washed three times with distilled water. Healthy seeds were selected and sown in plastic pots containing sand. Hoagland's nutrient solution with three different salinity levels i.e., 0, 60 and 120 m*M* NaCl was used in the present study. The nutrient solution was replaced after every three days during the entire experimental period. The experiment was laid out in completely randomized design (CRD) with nine replications for each experimental unit. Salicylic acid was applied exogenously with sprayer using three levels 0, 25 and 50 mg L^{-1}.

The plants were allowed to grow for 28 days after germination. Fresh weight of each plant was taken with the help of electric balance in grams. Dry weight of whole plant was observed after keeping in an oven at 70±2^{o}C for three days. For ion analysis dried ground material was digested in concentrated (H_2SO_4) and H_2O_2 as described by Wolf, (1982). The aliquot was filtered and used for determination of K^+, Na^+ and Ca^{2+} with the help of Flame-Photometer (PFP-7, Jenway, UK).

Analysis of variance for the data from each attribute was calculated using the COSTAT Computer Program. The Student New Multiple Range test at 5% level of probability was used to test the differences among mean values.

Results

Addition of excessive salt in the growth medium had highly significant adverse effect on shoot fresh biomass. Exogenous supply of both levels of salicylic acid was not beneficial in reducing the negative effects of salinity on shoot fresh biomass production (Fig. 1). Salt untreated plants showed 20 and 29% greater biomass than the plants treated with 60 and 120 m*M* NaCl salinity, respectively. Sorghum line, YSS-9 produced 10% higher shoot fresh biomass than that of PARI-S-4. The salt treatments showed highly significant effect on the shoot dry matter. Plants growing under control produced 28% more dry mass than the plants treated with 60 m*M* NaCl, and 32% greater shoot dry mass than that of growing under 120 m*M* NaCl. Exogenous supply of salicylic acid was not helpful in alleviating the adverse effects of salinity in salt treated plants. The two sorghum lines exhibited highly significant differences for this attribute (Fig. 1). The sorghum line, YSS-9 showed 24% greater shoot dry matter than that of the PARI-S-4.

Salt treatments influenced root fresh biomass adversely and plants grown at normal salinity level (control) produced 24% more root fresh biomass than that of treated with 60 m*M* NaCl and 29% greater than plants treated with 120 m*M* NaCl salinity. The exogenous supply of salicylic acid showed non-significant effects on root fresh biomass both in salinity treated and untreated plants (Fig. 1). Sorghum line, YYS-9 showed 13.48% high root fresh weight than that of PARI-S-4. Presence of excessive salts in the growth medium adversely affected the root dry biomass production in both sorghum lines. Control plants produced 27% more root dry biomass than the plants treated with 60 m*M* NaCl salinity and 32% than that of the plants grown under 120 m*M* NaCl salinity. The exogenous supply of salicylic acid showed non-significant effects on plant root dry biomass (Fig. 1). Sorghum line, YSS-9 showed 11% higher root dry biomass than that of PARI-S-4.

Root/shoot fresh and dry mass ratio remained unaffected by increasing salt levels in the growth medium (Fig. 2). Exogenous supply of salicylic acid also showed almost non-significant effects on the root/shoot fresh and dry biomass ratio. Excessive salts of the growth medium had highly significant effects on shoot and root Na^+ (Fig. 3). The plants treated with 60 m*M* NaCl salinity showed 59% more shoot Na^+ than the untreated plants and those grown under 120 m*M* NaCl salinity showed 73% higher Na^+ accumulation in their shoot than the salinity non-treated plants. Exogenous supply of salicylic acid had no role in reducing the excessive accumulation of Na^+ in the shoot/root of salt treated plants Addition of excessive salts in the growth medium adversely affected the shoot /root K^+ (Fig. 2). Salinity untreated plants accumulated 24.70% and 41.96% more K^+ in their shoot than the plants treated with 60 and 120 m*M* NaCl salinity, respectively. Exogenous supply of salicylic acid showed non-significant effects on this biochemical attribute.

Increasing salinity levels significantly decreased the accumulation of Ca^{2+} in both sorghum lines. Exogenous supply of salicylic acid also had significant effects on this biochemical attribute (Fig. 3). Overall, plants treated with 25 mg L^{-1} salicylic acid showed 13% more shoot Ca^{2+} than that control plants. The plants treated with 50 mg L^{-1} salicylic acid showed 9% more Ca^{2+} as compared with untreated plants. Plants of sorghum line, PARI-S-4, showed a gradual increase in root Ca^{2+} with the increase of exogenous supply of salicylic acid from 0-50 mg L^{-1}, when under high level of salt stress (Fig. 3). In contrast, plants of sorghum line, YSS-9, under salt stress remained almost unaffected by exogenous supply of salicylic acid.

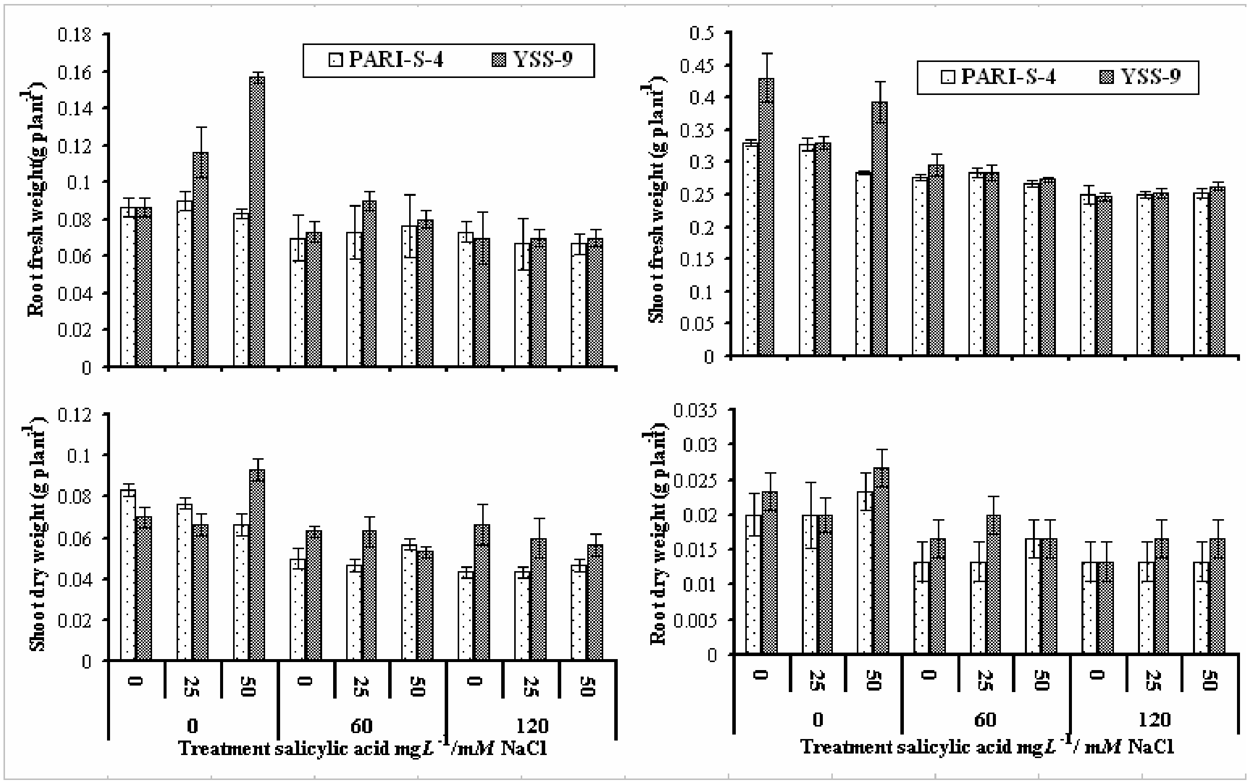

Fig. 1. Root and shoot fresh and dry weight of sorghum (*Sorghum bicolor* L.) cultivars influenced by different concentrations of exogenously applied Salicylic acid under different concentrations of NaCl stress (*n*=3).

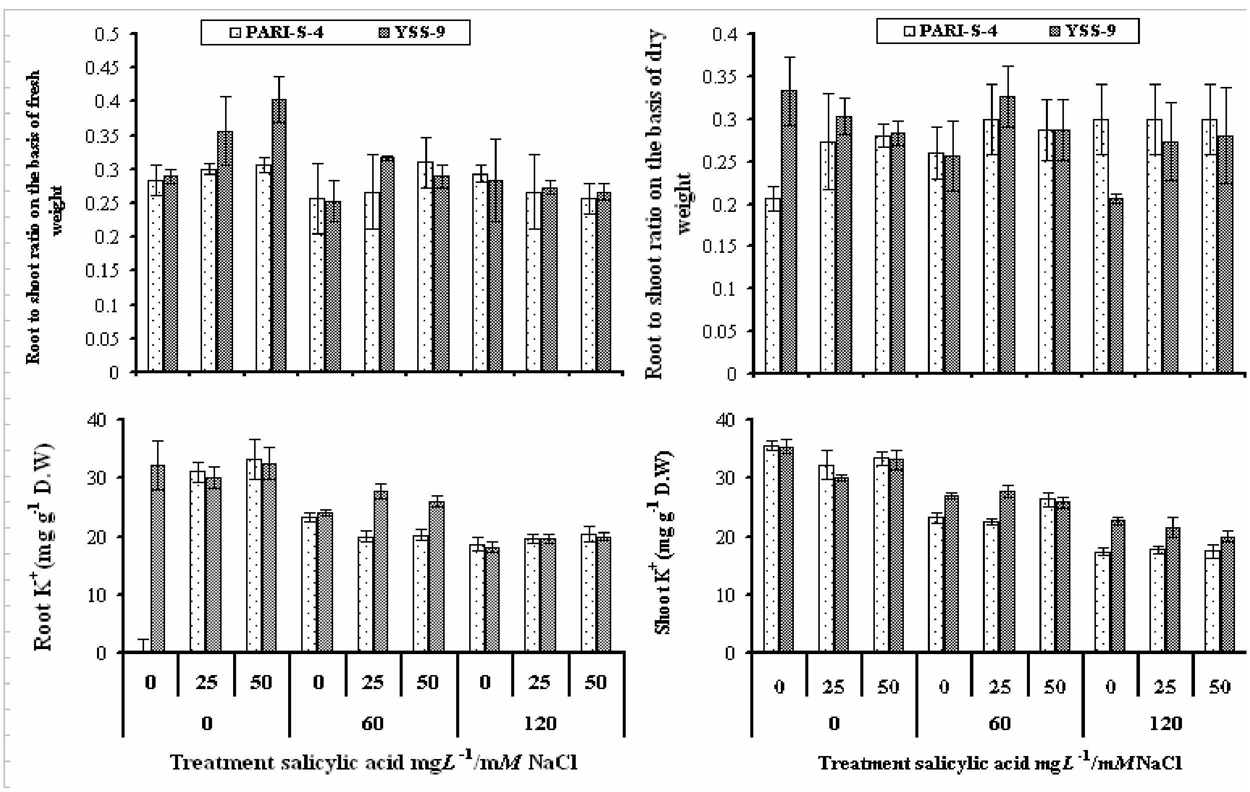

Fig. 2. Root to shoot ratios on fresh and dry weight basis and K^+ contents in root and shoot of sorghum (*Sorghum bicolor* L.) cultivars influenced by different concentrations of exogenously applied Salicylic acid under different concentrations of NaCl stress (*n*=3).

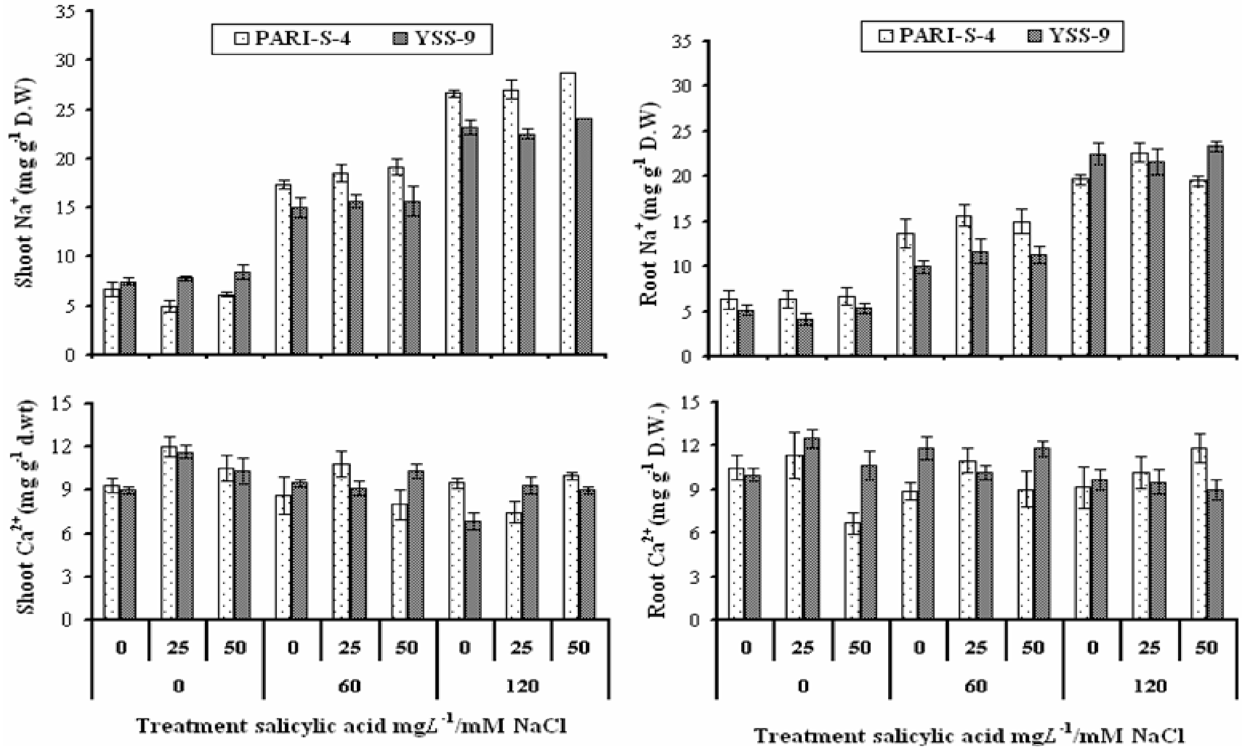

Fig. 3. Na^+ and Ca^{2+} contents in root and shoot of sorghum (*Sorghum bicolor* L.) cultivars as influenced by different concentrations of exogenously applied Salicylic acid under different concentrations of NaCl stress (*n*=3).

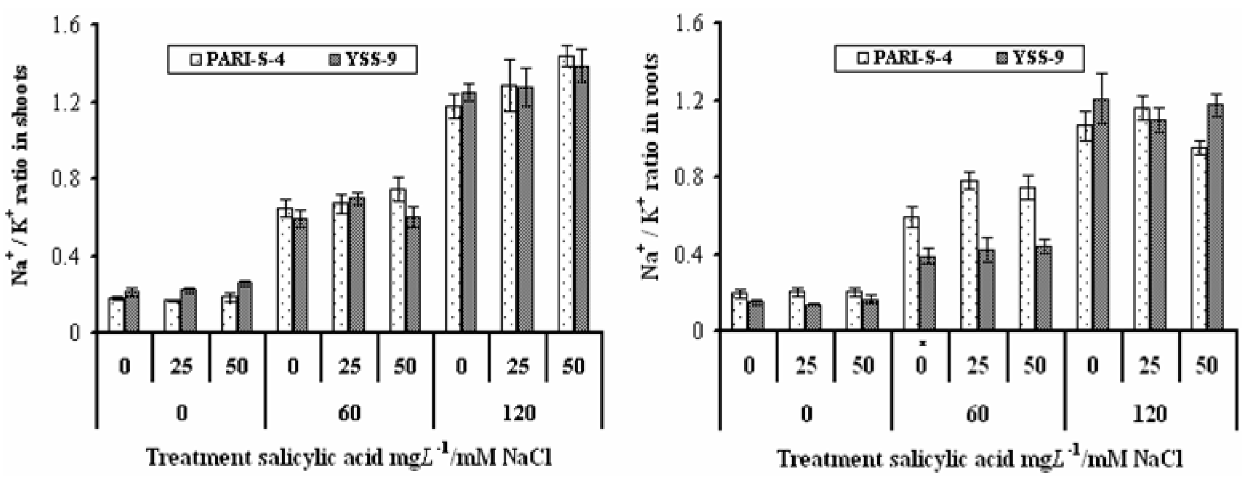

Fig. 4. Na^+/K^+ ratios in shoots and roots of sorghum (*Sorghum bicolor* L.) cultivars as influenced by different concentrations of exogenously applied Salicylic acid under different concentrations of NaCl stress (*n*=3).

Increasing salinity levels increased the shoot Na^+/K^+ ratio (Fig. 4). Plants treated with 60m*M* salinity showed 67.69% more shoot Na^+/K^+ ratio than the control while plants treated with 120mmol showed 83.84% higher Na^+/K^+ ratio than that of untreated plants. Exogenous supply of salicylic acid had no effects on this attribute. Salts of the growth medium had highly significant effects on root Na^+/K^+ ratio (Fig. 4). Exogenous supply of salicylic acid had non-significant effects on this biochemical attribute. Sorghum line, PARI-S-4 showed 6.55% more root Na^+/K^+ ratio than the YSS-9.

Discussion

Reduction in plant biomass production is a common phenomenon under salt stress. There are a large number of reports indicating the adverse effects of salinity on biomass production in different crop plants (Meloni *et al*., 2001). Almost similar trend was observed in the present investigation. The maximum plant biomass production was observed in control plants, a sharp decline in plant biomass production was recorded in salinity treated plants, particularly those subjected to higher salinity levels. Exogenous supply of salicylic acid, however did not contribute in reducing the effect of salinity on fresh biomass production. There are, however, some contrasting reports (Khodary, 2004; Hamid *et al*., 2007) indicating significant role of exogenously applied salicylic acid in alleviating the negative effects of salinity in crop plants. The difference in the results of the present study and the above-mentioned report may be due to differences in plant species/lines used for experimentation.

Presence of excessive salts in the growth medium reduces the dry matter of plant (Gaballah & Gomma, 2004), high level of the salinity also reduces the root growth (Ashraf *et al.,* 2002; Zahid *et al*., 2002). Almost similar response to salinity was observed for dry mass production in both sorghum lines, during the present study. Reduction in dry mass production was maximum in plants grown at 120 m*M* NaCl salinity levels, followed by the plants grown at 60m*M* NaCl salinity level and untreated plants.

In general, plants showing less reduction in fresh and dry mass production under salt stress are considered more salt tolerant. Hence, sorghum line, YSS-9 is more salinity stress tolerant than that of PARI-S-4. It is reported that high concentration of salts in growth medium proved that increase in plant root/shoot ratio (Meloni *et al*., 2001). Present investigation showed salinity did not affect the root/shoot fresh and dry biomass ratio. Exogenous supply of salicylic acid also showed non-significant effects on the root/shoot ratio of fresh and dry biomass.

Salinity limits the plant growth causing hyper ionic and hyper osmotic stress effects (Rout & Shaw, 2001; Rao *et al*., 2002). Excessive amount of salts adversely effects the plant growth and different biochemical and physiological process (Brugnoli & Lauteri, 1991; Dionisio-Sese & Tobita, 2000). Salinity also alters the accumulation of different ions in crop plants (Ahmad *et al*., 2002). In the present investigation an increase in Na^+ accumulation in root and shoot of both sorghum lines were observed due to salinity stress. The decreased Na^+ accumulation in YSS-9 at high salinity levels can be explained by its high salt tolerant ability (better growth and biomass production). There are some other reports (Chhipa & Lal, 1995; Iqbal *et al*., 2006) indicating that varieties/lines with reduced Na^+ accumulation at high salt levels are more tolerant. Some earlier studies indicated that exogenous supply of salicylic acid reduces the excessive accumulation of toxic ions especially Na^+ in different crop plants (Afzal *et al*., 2005). In contrast to this, non-significant effect of salicylic acid in reducing the negative effects of salinity on Na^+ accumulation was observed during the present study.

It is well reported that salinity decreases the K^+ accumulation in different crop plants (Ahmad *et al*., 2002; Iqbal *et al*., 2006). In the present investigation, a decrease of shoot/root K^+ in both sorghum lines was recorded. Exogenous supply of salicylic acid showed no effect on the shoot/root K^+. Exogenous supply of salicylic acid however, increased the accumulation of Ca^{2+} in the shoot of both sorghum lines. Na^+/K^+ ratio is considered as the basis of salt tolerance in plants and increases with increase of salinity level (Hasegawa *et al*., 1986; Chhipa & Lal, 1995). Almost similar trend was observed in present investigation, Na^+/K^+ ratio increased with increase of salinity level. Exogenous supply of salicylic acid did not contribute in reducing the effect of salinity on Na^+/K^+ ratio.

It was observed that salinity had greater effect on the plant growth and increase of salinity lead to the decrease of shoot/root fresh and dry mass production; as a result the overall plant growth was effected. However, exogenous supply of salicylic acid (25 and 50 ppm) showed almost non-significant effects. Sorghum line, YSS-9 proved to be more salt tolerant than PARI-S-4, producing greater biomass and accumulating less Na^+ and more K^+ and Ca^{2+} under salinity stress.

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