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Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.)

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Abstract

Salinity is worldwide problem that limits the growth and productivity of all vegetations and it is going to increasing day by day. The objective of the investigation was to study the response of okra (Abelmoschus esculentus L.) in terms of morphological and physiological characteristics under salt stress conditions. Okra seeds of cultivar Chinese Red were grown in plastic pots having fine sand as growth medium. After 30 days of germination, the plants were subjected to salt stress under control, 25, 50, and 75 mM NaCl. Increasing salinity caused a decrease of germination percentage, shoot and root length, plant height, pod weight, pod length, photosynthesis rate, and stomatal conductance. The Na^+ and Cl^- accumulation were correlated with a decline in concentration of K^+ in leaves and roots. Under salinity, a high concentrations of Na^+ and Cl^- were noted in both leave and roots portions.

Key words: Okra, NaCl, salt stress, photosynthesis rate, stomatal conductance

Introduction

Abiotic stresses like heat, cold, drought and salinity affect the plant growth and productivity (Allakhverdiev *et al*., 2000) but the salt stress exerts more drastic effects in terms of low productivity (Munns, 2002). The salt affected area is increasing day by day and total salinity affected area is about 831 x 10^6 ha (Beltran and Manzur, 2005). It has been estimated that one-half of all the irrigated land (about 2.5 x 10^8 ha) are severely affected by salinity (Rhoades and Loveday, 1990).

High salt contents reduce the growth and production by affecting physiological processes, including modification of ion balance, water status, mineral nutrition, stomatal behaviour, and photosynthetic efficiency (Munns, 1993). Most plants are salt sensitive with either a relatively low salt tolerance or severely inhibited growth at low salinity levels so differ in the growth response to salinity (Moisender *et al.*, 2002; Sheekh and Omer, 2002). Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress (Murphy and Durako, 2003). High concentration of salts in the root zone decreases soil water potential and the availability of water (Lloyd *et al*., 1989). This deficiency in available water under saline condition causes dehydration at cellular level and ultimately osmotic stress occurs. The excessive amounts of toxic ions like Na^+ and Cl^- create an ionic imbalance by reducing the uptake of beneficial ions such as K^+, Ca^{2+}, and Mn^{2+} (Hasegawa *et al*., 2000).

The higher ratios of toxic salts in leaf apoplasm lead to dehydration and turgor loss, and death of leaf cells and tissues (Marschner, 1995). Salt stress has various effects on plant physiological processes such as increased respiration rate and ion toxicity, changes in plant growth, mineral distribution, membrane instability resulting from calcium and potassium displacement by sodium (Grattan and Grieve, 1992), membrane permeability (Gupta *et al*., 2002), and decreased efficiency of photosynthesis (Ashraf and Shahbaz, 2003; Kao *et al*., 2003; Sayed, 2003). The most important process that is affected by salinity is photosynthesis (Hayat *et al.,* 2010). Reduced photosynthesis under salinity is not only attributed to stomatal closure leading to a reduction of intercellular CO_2 assimilation, but also to non-stomatal factors like reduction in green pigments and leaf area. There is increasing evidence that salts affect photosynthetic enzymes, chlorophylls and ionic contents (Misra *et al*., 1997).

Okra (*Abelmoschus esculentus* L.) is recognised as annual herbaceous plant grown in tropical and subtropical areas and serves as a source of carbohydrates, fats, vitamins and various minerals (Oyenuga, 1968). In spite of having good nutritional value, it's per hectare yield is very low. This decline in optimum yield is due to the drastic effects of salts, which are deposited in soil by the use of brackish underground water, and addition of industrial effluents in our canals also adds salts in irrigation water. This application of saline water reduces the transpiration and causes an imbalance in evapo-transpiration rate and induces

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the reduction in yield (Dudley *et al.*, 2008). High ratios of salts in root zone affect different processes like root density, root turgor pressure, and its growth, and ultimately create hindrance in water absorption (Maggio *et al.*, 2004). Okra plant at earlier growth stages is more sensitive to salinity (Cedra *et al.*, 1982), as it affects water relations and nutrient uptake of plants. While later on, the ionic stress inturn reduces leaf expansion. During long term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves and thus reduction in photosynthetic rate is a common observation (Cramer and Nowak, 1992). Salinity changes photosynthetic parameters (Nadeem *et al.,* 2006), including osmotic and water potential (Azevedo-Neto *et al.,* 2004), transpiration rate (Karlberg *et al.,* 2003), leaf temperature (Maricle *et al.,* 2007), and relative leaf water content (Lee *et al.,* 2005). The aim of present study was to analyze the drastic effects of salt stress on some morphological and physiological attributes of okra plant.

Material and methods

Plant material and growth conditions

Okra (*Abelmoschus esculentus* L.) was used for this study. The experiment was carried out in net house. Seeds of okra were allowed to germinate in 9 L plastic pots filled with fine sand as growth medium. Ten seeds per pot were sown but after 15 days of germination, the plants were thinned out to five. The experiment was carried out with three replications. Plants were grown in Hoagland (Hoagland and Arnon, 1950) solution under non saline conditions for 30 days after germination. Afterwards, the salt treatment was initiated. Sodium chloride was dissolved in distilled water to obtain final concentration of, 0 (Control), 25, 50 and 75 mM and then these solutions of different NaCl concentrations were applied to create the salinity while half strength Hoagland solution was applied as nutrient medium . To prevent the plants from osmotic shock, the NaCl concentrations were imposed in 25 mM increments every day until final concentrations were reached after three days interval. Plants were grown for 15 days under salt stressed conditions. Irrigation along with half strength Hoagland solution was applied to the selected treatments according to the need of the plants by regularly observing the wetness extent of sand. Seed germination was calculated separately in Petri dishes by applying the respective concentration of NaCl solution on filter papers.

Morphological attributes

The seeds were surface sterilized with sodium hypochlorite 0.5 % (v/v) for 20 min., washed repeatedly with distilled water and then sown in Petri dishes having filter papers moistened with respective saline solution in three replications (10 seeds each Petri dish). After five days the number of seeds germinated in each Petri plate was counted. The germination percentage was calculated as under:

Germination percentage =
(No. of seeds germinated/ No. of seeds sown) × 100

The shoot length of six randomly selected plants (two from each replication) was measured before the salinity (application and at the time of harvesting, and the difference was taken as equal to the shoot length/elongation under saline condition) in centimeters from the base to the top of shoot with the help of measuring rod. The root length of six randomly selected plants (two from each replication) was measured in centimeters, with measuring tape, from the base to the tip of longest root. The final height of the plant was recorded in centimeters (cm) from the ground level to the apical bud at maturity stage when apical growth of the main stem ceased. The plant height included final shoot length + final root length at the time of plant harvesting. For shoot elongation the shoot lengths (above ground plant for 4 plants per pot) were noted before the salinity application and the difference between this reading and final plant height represented the shoot elongation.

Shoot elongation = Plant height at the time of harvesting –
Shoot length before salinity application

The marketable green pods were weighed and average pod weight was calculated for each plant by dividing the total plant yield with respective number of pods per plant. The average pod weight (g) per plant was worked out with digital balance for statistical analysis. The length of each of the picked pod was measured with vernier calipper and average pod length was worked out.

Physiological attributes

Photosynthesis rate (Pn) and stomatal conductance (g_s) and transpiration rate (E) were recorded on intact leaves using an infra-red gas analyser (Analytical Development Company, Hoddesdon, England). Measurements were performed from 9.00 to 11.00 a.m. after 15 days of salinity application, with the following specifications/adjustments: molar flow of air per unit leaf area, 403.3 mM m^{-2} s^{-1}; atmospheric pressure, 99.9 kPa; water vapor pressure into chamber ranged from 6.0 to 8.9 mbar (photosynthetically active raditation) at leaf surface was maximum up to 1711 (mol m^{-2} s^{-1}) because this reading was shown by IRGA (infrared gas analyzer) on its screen at the time of use, temperature of leaf ranged from 28.4 to 32.4 ^{o}C, ambient temperature ranged from 22.4 to 27.9 ^{o}C, ambient CO_2 concentration was 352 mol mol^{-1}. The digested samples of root and leaf of okra were analyzed for Na^+ and K^+ by

Flame photometer (Jenway PFP-7, UK). A series of standards (ranging from 10 to 100 mg L^{-1}) of Na^+ and K^+ was prepared and standard curves were drawn. The values of Na^+ and K^+ from flame photometer were compared with standard curve and original quantities were computed. The chloride contents were measured with chloride analyzer.

Statistical analysis

The experiment was arranged in completely randomized design with two factor factorial arrangement, having three replications. The data recorded were analyzed statistically using Fisher' s analysis of variance technique and Duncan's Multiple Range test at 5 % probability level to compare the differences among treatment means (Steel *et al*., 1997).

Results

The salt stress exerted a significant ($P < 0.05$) decrease in germination percentage (Figure 1a). Maximum reduction in germination was observed at 75 mM as compared to the control. Maximum germination percentage (98.05%) was recorded at lowest salinity level (25 mM) while the lowest values (69.64%) for germination percentage was observed at highest salinity level of 75 mM. Reduction in shoot length was observed with the increase in salt stress (Figure 1b). It decreased continuously with the increment in salinity from control to 75 mM. The control showed the highest (23.56 cm) shoot length followed by 25 mM (21.45 cm), 50 mM (15.67 cm) and 75 mM (11.89 cm). Salt stress also exerted a drastic effect on root growth and development by affecting its length. All the three salinity levels (25, 50 and 75 mM) caused reduction in root length of okra plant as compared to the control (Figure 1c).

The plants grown under control (no salinity application) exhibited the maximum values (10.11 cm) for root length as compared to remaining treatments, which indicated that salinity is responsible for reduction in root length.

The plants grown under 75 mM salt stress showed the lowest values (8.44 cm) of root length. A significant ($P < 0.05$) reduction in plant height was also noted in all the plants grown at different salinity levels (Figure 1d). Maximum plant height (45.67 cm) was observed in plants grown under control while 25, 50 and 75 mM NaCl salinity levels exhibited the values for plant height as 41.98, 34.02 amd 26.33 cm, respectively. So, it is clear that highest salinity level 75 mM exerted the maximum drastic effects on plant height as compared to the other salinity levels. The salinity also influenced the yield of okra by affecting both the pod weight as well as pod length. The pod weights of all the plants grown under salinity represent the decrease as compared to the control (Figure 2a). But maximum decrease was recorded at 75 mM followed by 50 and 25 mM. The plants grown under 75 mM showed the minimum (5.39 g) as compared to the non saline control with highest pod weight (7.06 g).

The salinity levels 25 and 50 mM showed the moderate pod weight values of 6.76 and 5.98 g, respectively. Similarly decline in pod length was observed at all salinity levels with maximum reduction at 75 mM and minimum reduction at 25 mM salt stress while control exhibited the maximum pod length followed by 50 *mM* (Figure 2b). The photosynthesis rate and stomatal conductance are also influenced by salinity in all the plants grown under saline environment. The highest salinity (75 mM) caused the maximum reduction in both photosynthesis rate (Figure 2c) and stomatal conductance (Figure 2d) as compared to the low salinity (25 mM).

Maximum photosynthsis rate (39.67 µmol CO_2 m^{-2} S^{-1}) was noted at control (non saline) while other salinity levels, 25, 50 and 75 mM showed 31.78, 29.89 and 22.45 µmol CO_2 m^{-2} S^{-1}, respectively. So it is clear that high salt stress (75 mM) exhibited the maximum drastic effects in terms of reduction in photosynthesis rate. The stomatal conductance was also significantly ($P < 0.05$) influenced under the effect of salt stress. A decreasing trend was noted in case of stomatal conductance in response to salinity. All the salinity treatments showed significant ($P < 0.05$) variations for stomatal conductance. The plants grown under control (non saline) and 25 mM exhibited the maximum values (72.56 and 66.72 mMol m^{-2} S^{-1}, respectively) for stomatal conductance as compared to the remaining two salinity levels of 50 and 75 mM with values 61.78 and 54.88 *mM*ol m^{-2} S^{-1}, respectively. Na^+ and Cl^- tended to increase with the increment in salinity. So, maximum concentration of these ions was recorded at higher salinity level of 75 mM (Figure 3a and 3b).

Maximum amounts of sodium were noted in roots while maximum concentration of chloride was measured in leaves. On the other hand, salt stress caused a decrease in K^+ contents both in leaves and roots but maximum concentration was recorded in leaves as compared to the roots. The plants grown under control showed no significant difference in K^+ contents of both leaves and roots while the plants under 50 mM were also statistically similar for K^+ contents in their roots and leaves. However, the 25 and 75 mM treatments exhibited the prominent difference in K^+ contents of their leaves and roots (Figure 3c).

Discussion

The germination of the okra was affected because high salt concentration in the solutions sprayed on filter papers in

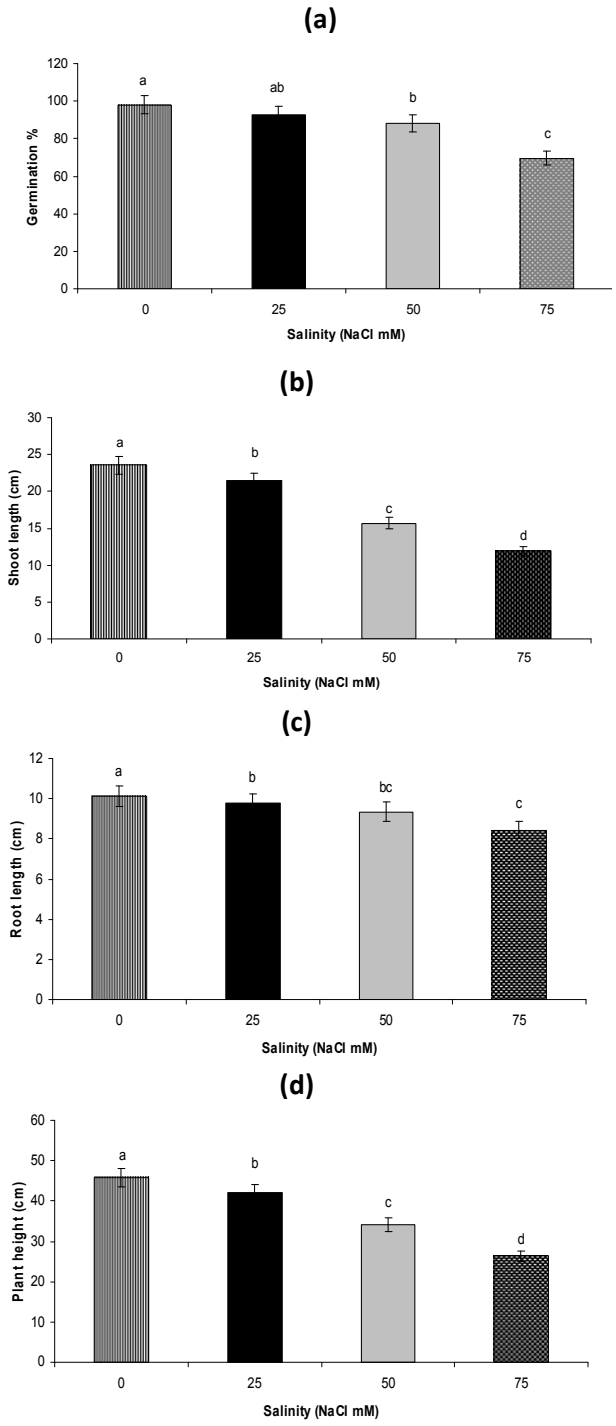

Figure 1: Effect of NaCl salt stress, germination %age, shoot length, root length and plant height of okra

Columns followed by different letter(s) is/are significantly different by DMRT at 5% level of significant
Error has ± S.E. for n = 3

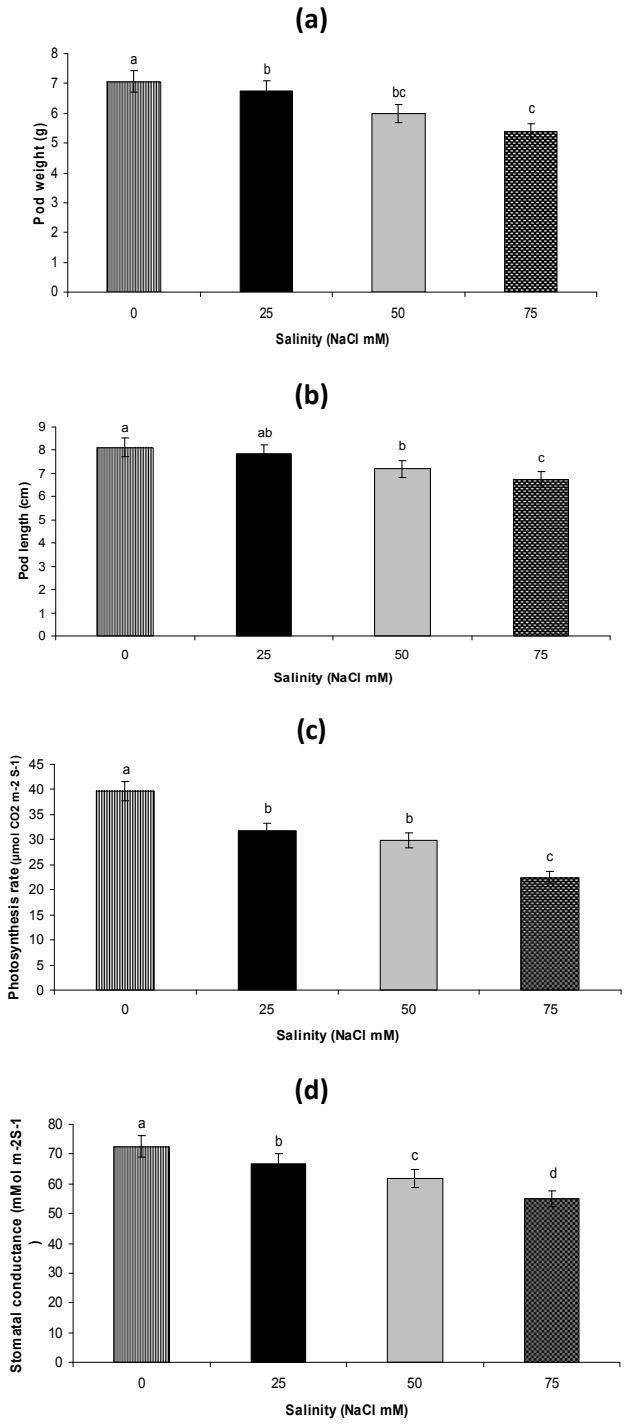

Figure 2: Effect of NaCl salt stress on pod weight, pod length, photosynthesis rate, stomatal conductance of okra

Columns followed by different letter(s) is/are significantly different by DMRT at 5% level of significant
Error has ± S.E. for n = 3

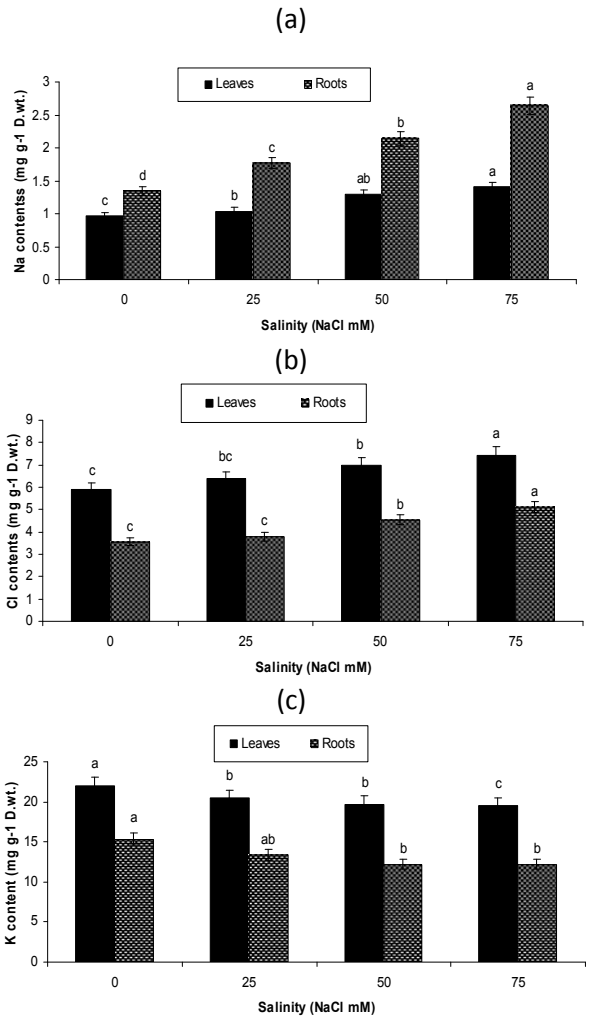

Figure 3: Effect of NaCl salt stress on sodium, chloride, and potassium contents in leaves and roots of okra

Columns followed by different letter(s) is/are significantly different by DMRT at 5% level of significant
Error has ± S.E. for n = 3

Petri dishes increases the osmotic potential which causes the seeds to use more energy to absorb water from the wet filter papers and hence affects the germination (Kafi and Goldani, 2001; Jamil and Rao, 2004). The depressed growth of plants may be due to the toxic effect of Na^+ and Cl^- ions present in NaCl and low water potential in the rooting medium (Silveira *et al.,* 2009). It is reported that salt stress affects the plant growth and development by influencing fresh and dry weights of roots, shoots along with shoot length (Ashraf *et al*., 2003). In the present study, the plants exhibited the lowest germination percentage (69.64%) under 75 mM NaCl salinity as compared to the other treatments and it may be due to the increase in osmotic potential. It has been suggested that plants having longer roots in non-saline conditions had shorter roots in saline conditions (Figure 1c). Growth attributes like plant height, shoot elongation, shoot and root length were severely decreased with salinity. It was noted that plants growing under saline condition remained stunted. The lower water potential (ψ_w) in saline soil in turn lower cell tugor causing reduction in cell elongation and cell division (Greenway and Munns, 1980). Plant growth is important character in determining the salt tolerance ability of the plants. Although plant height is genetically controlled, environmental factors also have strong influence in the expression of genes as it is very clear from the current work that all treatments of salt stress significantly influenced the plant (Figure 1d). The okra plants under normal and low salinity level (25 mM) may have adjusted osmotically to the growing conditions as a result of which they were successful in maintaining required cell enlargement so showed the maximum plant height (Figure 1d) along with root/shoot lengths as compared to the higher salinity levels (50 and 75 mM). The plants grown under high salinity (75 mM) fail to activate the dehydration avoidance mechanism like making root membranes immpermeable for toxic ions of Na^+ and Cl^- so plants did not maintain stomatal conductance (Figure 2d) up to desired rate (Abbruzzese *et al.,* 2009), thus could not with-stand high salt stress and experienced the reduction in growth. Roots are directly in contact with growth media containing toxic salts that stop the long term root growth (Tyerman and Skerrett, 1999) which indirectly affect the biomass production. Under saline condition, CO_2 assimilation of plant become decreased, it is major energy source for growth and development, so, ultimately root growth decreases (Syvertsen *et al.,* 2000; Kasukabe *et al.,* 2004, 2006). The reduction in root length caused the decrease in biomass which is commonly observed under salt stress (Vasquez *et al.,* 2006; Keutgen and Pawelzik, 2008). A decrease in root length with the increments in salinity in present assessment confirms the results of Syvertsen *et al.,* 2000; Kasukabe *et al.,* 2004, 2006; Ibrahim *et al.,* 2007.

The okra plants grown under low salinity level (25 mM) might have developed osmotic adjustment mechanism, hormonal imbalance and ion exchange to alleviate the drastic effects of toxic ions, so, these plants exhibited better growth in terms of maximum root/ shoot length and height while under high salt stress (75 mM) they failed to develop the efficient osmotic adjustment mechanism and showed reduction in the above mentioned growth attributes. In the present study, rate of photosynthesis and stomatal conductance reduced with the rise in salinity (Figure 2c and 2d). Salinity severely affects the photosynthetic activity of plants and it may be because

of the variations in osmotic potential and water potential within the tissues. As the salts within the plant tissues increases osmotic potential increase while water potential decreases (Ashraf and Foolad, 2005), so, this decrease in water potential affects the opening and closing of stomata which finally causes the imbalance in gaseous exchange and disturbs the photosynthetic apparatus. On the other hand, reduction in photosynthesis may be due to reduction in stomatal conductance (Chartzoulakis *et al.,* 2002; Sudhir and Murthy, 2004). It is well documented that salinity affects growth and leaf water potential, stomatal conductance and transpiration rate (Parida and Das, 2005) for okra (Ikram, 2009). Under salt stress, tolerant plants maintain high concentrations of K^+ and low concentrations of Na^+ in the cytosol while in sensitive plants the ratio of Na^+ increases as compared to the K^+ and drastically affects the cell organelles. The plants regulate the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for ion transport (Zhu, 2003). A greater degree of salt tolerance in plants was found to be associated with a more efficient system for selective uptake of K^+ over Na^+ (Neill *et al*., 2002).

The selective uptake of K^+ in contrast to Na^+ was considered one of the important physiological mechanisms contributing to salt tolerance in many plant species (Poustini and Siosemardeh, 2004). Okra grown under high salinity (75 mM) accumulated maximum amounts of Na^+ in their leaves and roots, so, the growth of these plants was affected due to high concentration of Na^+ and low ratios of K^+ (Aydin *et al*. 2002; Ahmadi *et al*., 2009; Dashti *et al*., 2009). The salt tolerance in okra plants may be attributed to effective exclusion of Na^+ as was also discussed by Flowers and Yeo (1981), Yeo and Flowers (1984) and Sharma (1986) in rice. There was a decrease in K^+ concentration both in leaves and roots with increased NaCl salinity in the okra (Figure 3c). Maintenance of higher K^+/Na^+ ratio under low salt stress (25 mM) may be one of the reasons for superior growth (Ashraf and Ahmad, 2000). High levels of K^+ in young leaves are associated with salt tolerance in many plant species (Gorham, 1993; Storey *et al*., 1993; Khatun and Flowers, 1995).

It can be concluded that salt stress has affected the okra plant growth and development as well as its physiological process like photosynthesis rate and stomatal conductance. The effect can be attributed to antagonistic influence of Na^+ to K^+; it means Na^+ reduced the absorption of K^+ and this ion is very beneficial is stomatal conductance because it regulates the opening and closing of stomata, so, Na^+ indirectly affects the stomatal conductance by reducing the ratios of K^+/Na^+. On the other hand, higher ratios of K^+/Na^+ under salt-stress were calculated in leaves as compared to the roots.

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