Current Research Journal of Biological Sciences 2(1): 76-82, 2010
ISSN: 2041-0778

Submitted Date: October 30, 2009 Accepted Date: November 21, 2009 Published Date: January 05, 2010

Effect of saline culture on the concentration of Na^+, K^+ and Cl^- in *Agrostis tolonifera*

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Abstract: A glass house pot experiment was conducted to assess ionic (Na^+, K^+ and Cl^-) relations and contents in two differently adapted (salt marsh (SM) and inland (IL)) clones of *Agrostis stolonifera*. In non-saline medium the roots, stems and leaves of the SM and IL clones demonstrated relatively lower concentration of Na^+ and Cl^- as compared to concentration of K^+. But when cultured with NaCl, the concentration of Na^+ and Cl^- increased in all organs in both the clones. Increased NaCl concentration in culture medium caused a progressive decrease of K^+ uptake in the roots and stems of both the clones. It was also found that Na^+ and Cl^- concentrations were higher in stems than in roots and leaves in salt treated plants. In the leaves, however, Na^+ and Cl^- levels showed a stiochiometric relationship. In older leaves higher concentration of Na^+ and Cl^- was found as compared to younger leaves in SM and IL plants. Better adaptability of SM plants to salt stress was due to some inherent genetic variability linked with their growth.

Key words: *Agrostis stolonifera*, genetic variability, inland clone, NaCl, salt marsh clone and stiochiometric relationship

INTRODUCTION

All plants are subjected to multitude of stresses throughout their life cycle. The major environmental factor that currently reduces plant productivity is salinity (Rausch, 1996; Serrano, 1999). Reduced growth of plants is mainly due to the severe effects of salinity on various biochemical and physiological processes and this was mainly due to the salt induced osmotic and toxic effects which minimize the uptake of other mineral nutrients such as N, K^+ and Ca^{+2}, from the rooting medium (Ashraf, 2004). The ion concentrations in roots, shoots and leaves are maintained by regulating ion transport to acclimatize salt stress (Tester and Davenport, 2003).

The plants have adopted strategies to maintain K^+/ Na^+ ratios in the cytosol such as regulation of K^+ uptake and/or prevention of Na^+ entry, Na^+ efflux from cells and its utilization for osmotic adjustments. The Na^+ compartmentalization to vacuoles or accumulation of compatible solutes would establish the osmotic homeostasis (Ashraf and Haris, 2004; Munns, 2005). At different stages of growth, plants differ in nutrient uptake and metabolism (Grattin and Grieve, 1999). The uptake of ground water by plant roots can increase the salinity of ground water or the soil around the roots due to the leaving of salts (Niknam and McComb, 2000). These variable conditions make research difficult, and this is compounded by the fact that each species has its own level of salt tolerance (Ashraf, 2004; Munns *et al*., 2006).

Unfortunately, most crops are not halophytic, and studies in crops suggest that salt tolerance is a multigenic trait (Niknam and McComb, 2000) which makes it more difficult to study and improve. The traits relating to salt tolerance of plants are generally associated with lower accumulation of Na^+ and higher accumulation of K^+ of the plants grown in the presence of salts (Flowers, 2004) which could ultimately be passed along to offspring (Niknam and McComb, 2000) but tolerance to salinity stress is not related to the concentration of sodium in the shoot in all cases (Cramer, 1994). Thus, considerably more research is needed to find out the basis of salt tolerance in plants, particularly in those naturally adapted to salt affected soils. The primary objective of this research was to determine the ionic relations and ionic contents (Na^+, K^+ and Cl^-) in different parts of salt marsh (SM) and inland (IL) clones of *Agrostis stolonifera* under varying salinity stresses.

MATERIALS AND METHODS

A pot experiment arranged in randomized design was conducted at University of Wales Swansea, UK and University of Gujrat, Pakistan to determine the ionic relations and ionic contents (Na^+, K^+ and Cl^-) of salt marsh and inland clones of *Agrostis stolonifera*. The plants were cultured for about three months in $1/5^{th}$ dilution of A and H solution. Plants were maintained in an automatically ventilated heated glasshouse with an 18 hour photoperiod. Temperatures were normally in the range of 20-25 ºC.

Tillers of standard (8-10 cm in length) from these stock plants were used for sub-culturing in modified A & H culture solution for about fourteen days. Rooting of *A. stolonifera* generally took place in two to three days. For

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the measurement of tissue ion content salt treatments of different levels (0, 100, 200 and 300 mM NaCl) were initiated after plants had been water cultured for 14 days.

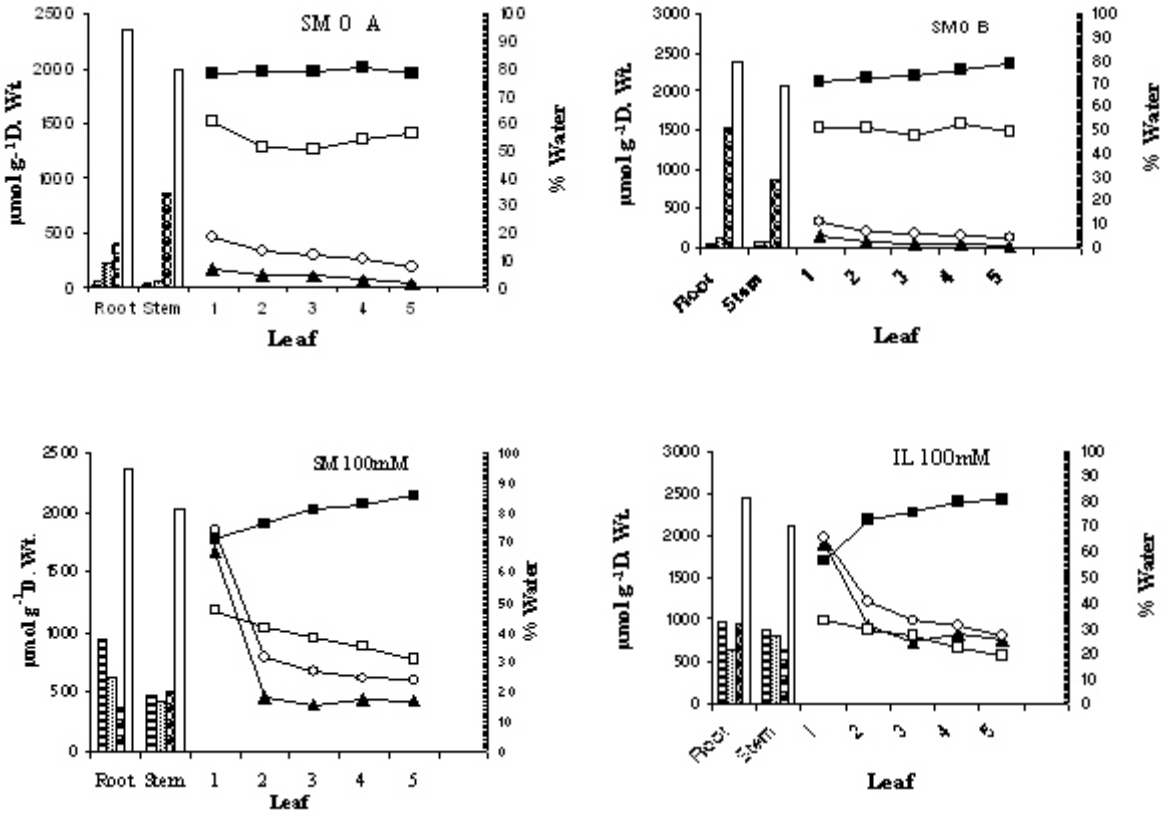
Oven dried roots, stems, individual leaves were weighed (0.1g) and ground to be digested with 2 ml of sulfuric acid-hydrogen peroxide mixture following Wolf (1982). Sodium and K+ in the digests were determined with a flame photometer (Jenway Ltd. Felstsd, Dunmow, Essex, U.K.). Cl^- was determined with a chloride analyzer (Sherwood). The results of these analyses were expressed on a dry weight basis (μmoles g^{-1} d. wt.).

Analysis of Variance (ANOVA) technique was employed for carrying out statistical analysis of the data collected using Costat 6.3 computer package (Cohort Softwares, California, USA). The mean values were compared with the least significant difference test (LSD) following Snedecor and Cochran (1980).

RESULTS

Plants were treated with different salt treatments for two weeks and then data was scored on water contents (per fresh weight basis) and ionic concentrations (Na^+, Cl^- and K^+) (on a dry weight basis) for roots, stems and individual leaves of *A. stolonifera* (Fig. 1). In non-saline medium, the roots, stems and leaves of the SM and IL clones of *A. stolonifera* contained relatively high concentrations of K^+ but relatively low concentrations of Na^+ and Cl^- and an approximate stoichiometric relationship between the Na^+ and Cl^- content in leaves of *A. stolonifera* of both clones (Fig. 2). The roots of SM plants have similar but roots of IL plants have higher K^+ concentration than in the leaves but stems of both have K^+ concentrations intermediate between those of roots and leaves.

When plants were cultured with NaCl, Na^+ and Cl^- concentrations increased in all organs in both clones but IL clone had larger increase in the concentrations of these ions exceptionally the roots of SM plants have got high concentrations of these ions. In contrast, there was a progressive decrease in K^+ concentrations in roots and stems of both clones with a progressive increase in NaCl concentration in the culture medium. In leaves, however, K^+ concentrations were similar at all concentrations of NaCl although they had decreased relative to those in the leaves of plants grown in non-saline medium. Moreover, leaf K^+ concentrations remained higher than stem concentrations, which in turn remained higher than root concentration, in all salt levels, except with the IL in the 100 mol m^{-3} NaCl treatments where the root K^+



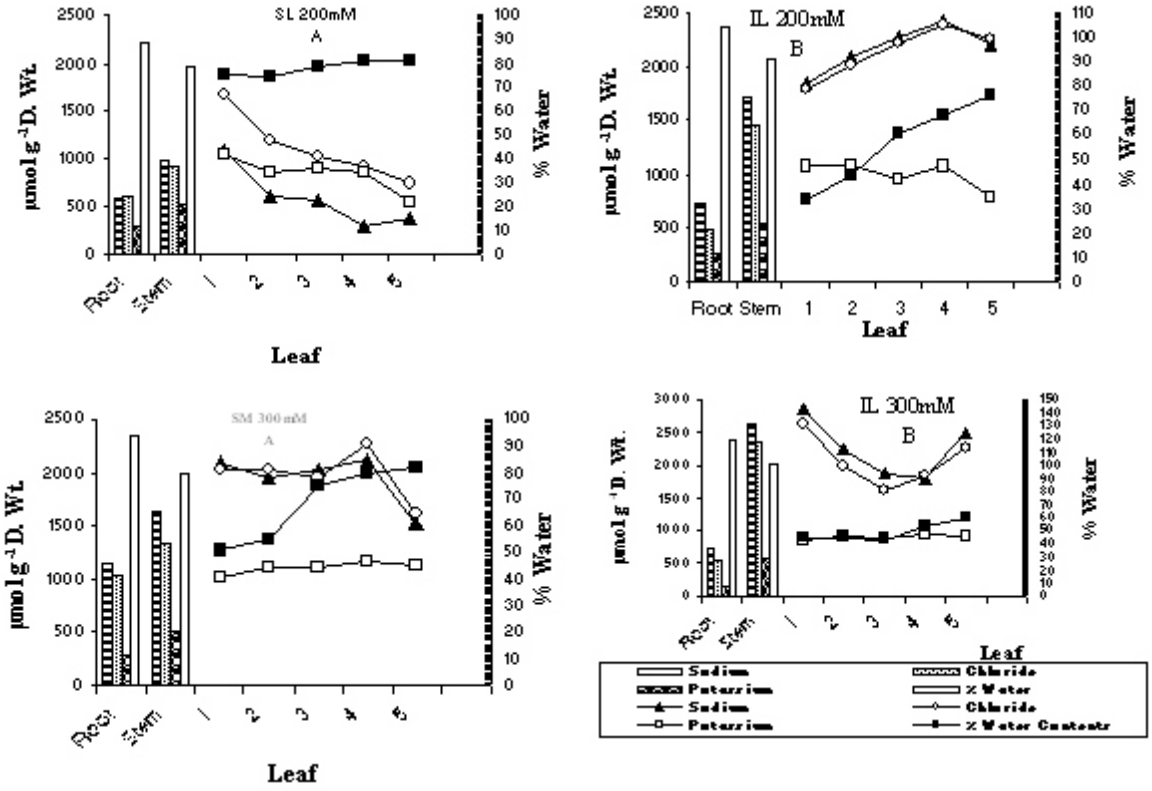


Fig. 1: Effect of different levels of NaCl (mol m^{-3}) on the concentration of Na^+, Cl^-, K^+ on tissue dry weight basis (µmol g^{-1} D wt.), water content (%) in different organs of plants

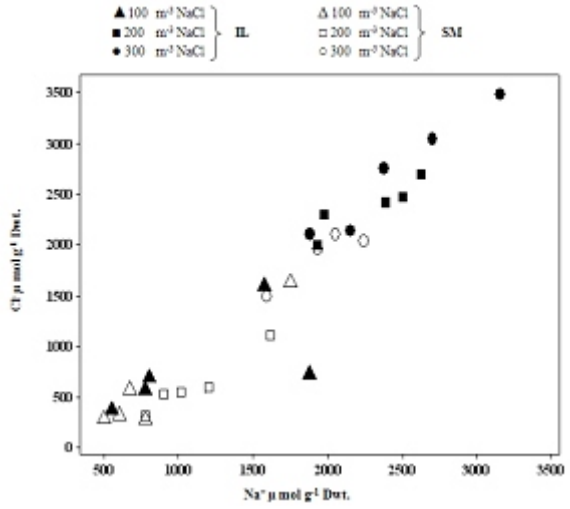


Fig. 2: Relationship between Na+ and Cl^- concentrations of the leaves of salt treated stolonifera

concentration was higher than the stem concentration. K^+ concentration in the older leaves of plants grown in 100 and 200 mol m^{-3} NaCl were higher than those in younger leaves while in the 300 mol m^{-3} NaCl treatment, K^+ concentrations were similar in all leaves.

Ionic concentrations (on a tissue water basis) for roots, stems and individual leaves from the various salt treatments after culturing the plants of *A. stolonifera* for two weeks were also determined (Fig. 3). In non-saline media the Na^+ and Cl^- concentrations were lower in roots and stems than in the leaves and the tendency of older leaves to have higher concentrations of Na^+ and Cl^- than in the younger leaves was maintained. In contrast to the dry weight basis the K^+ concentration on a tissue water basis showed distinct trends. Roots showed lower concentrations of K^+ than stems which in turn showed lower concentrations than leaves and the older leaves had got higher K^+ concentrations than those of the younger leaves. Cl^- concentrations of the leaves of the SM plants were higher than the leaves of the IL plants, whereas the Na^+ concentrations of the leaves of both clones were similar. K^+ concentrations of the leaves of the IL were higher than those of the leaves of the SM plants and water contents were the same as described in Fig. 1.

Culturing the plants with 100 mol m^{-3} NaCl, the roots and stems had lower Na^+, Cl^- and K^+ concentrations than leaves as older leaves accumulated higher ions than the younger leaves when water loss had occurred but the leaves of IL plants had got higher ionic concentrations than the leaves of the SM plants with much more pronounced difference in the oldest leaf (1700 and 3000

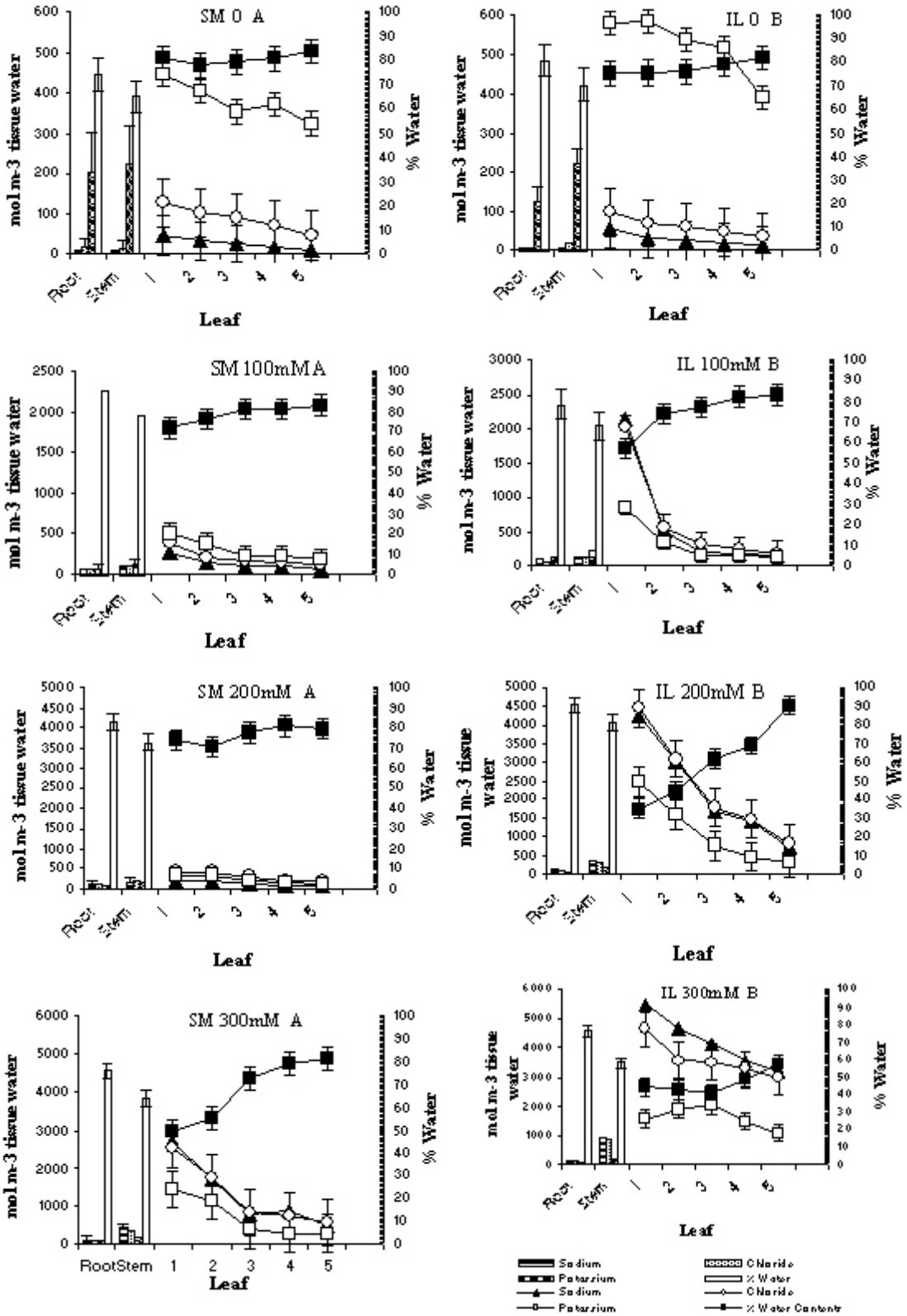


Fig. 3: Ionic concentrations of roots, stems and individual leaves of A. stolonifera clones on tissue water basis (mol m^{-3}) for various salt treatments

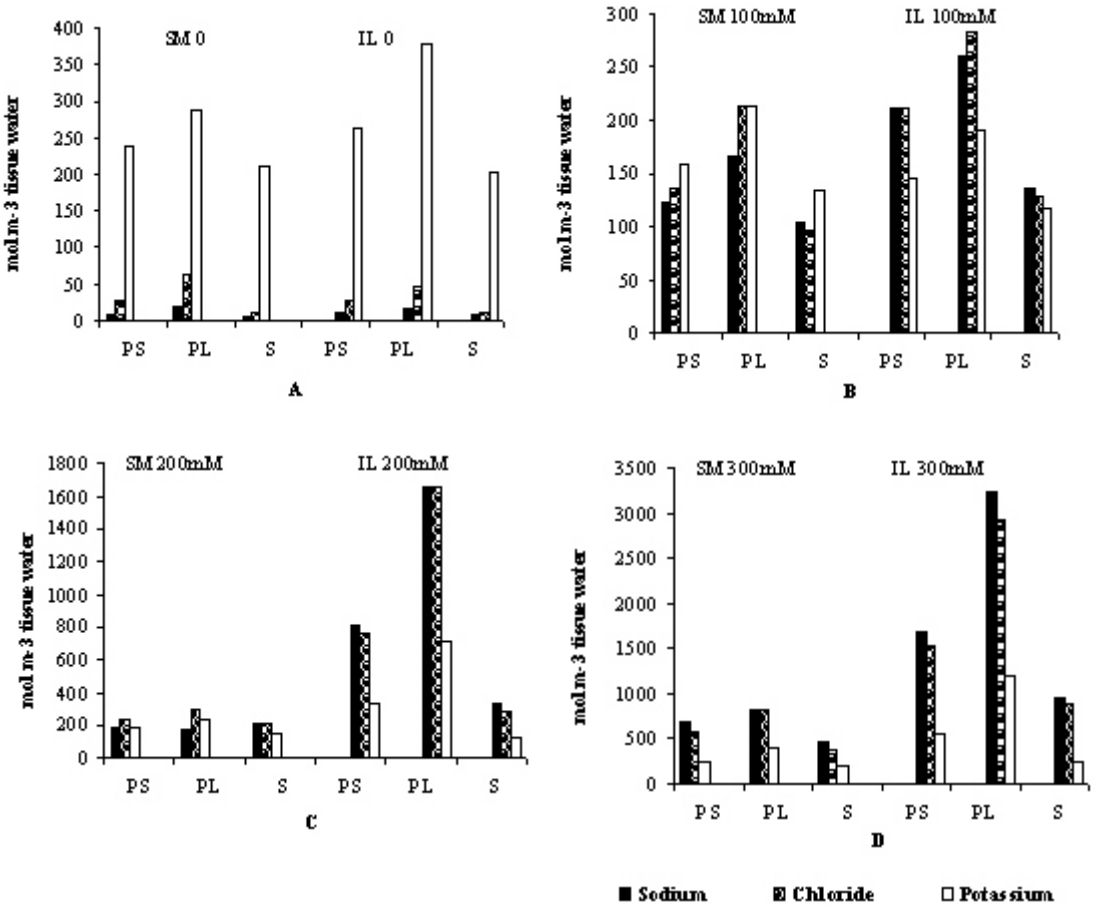


Fig. 4: Ionic concentrations (mmol m^{-3}) of pooled organs in *A. stolonifera*. PS: Pooled shoot; PL: pooled leaves; S: Stem; SM: Salt marsh clone; IL: Inland clone

mol m^{-3} Na^+ and Cl^-) but no marked difference was found among the younger leaves of both clones (Fig. 3). Stem Na^+, Cl^- and K^+ concentrations were higher than root ionic concentrations in both clones but IL plants have somewhat higher K^+ concentrations in stems. The oldest leaf of the IL had K^+ concentrations of 727 mol m^{-3} K^+, while the oldest leaf of the SM plants had concentrations of 452 mol m^{-3} K^+ on a tissue water basis whereas the younger leaves of the SM plants had higher K^+ concentrations than youngest leaves of the IL.

In 200 mol m^{-3} NaCl level once again Na^+, Cl^- and K^+ concentrations were higher in the older leaves than in the younger leaves in both clones (Fig. 3). However, there were considerable differences between the Na^+ and Cl^- concentrations of the oldest and the youngest leaves of the IL, but only small differences in the SM plants. The oldest leaves (leaf 1 and leaf 2) of the IL had Na^+ and Cl^- concentrations 4000 and 3000 mol m^{-3} tissue water, respectively, while the youngest leaf had 700 mol m^{-3} on a tissue water basis. In contrast the oldest leaf of the SM plants contained 350 mol m^{-3} Na^+ or Cl^- on a tissue water basis and the youngest leaf contained 150 mol m^{-3}. K^+ concentrations of the leaves of the SM plants remained similar to those of Na^+ and Cl^- concentrations whilst leaf 1 of the IL plants contained 2420 mol m^{-3} K^+ on a tissue water basis and the youngest leaf (leaf 5) contained 270 mol m^{-3} K^+. The root ion concentrations were lower than the stem concentrations in both clones. Stem Na^+ and Cl^- concentrations of the SM plants tended to be higher than those of the younger leaves whereas the concentrations in the stems of the IL plants were lower than those of the younger leaves. In contrast, the stem K^+ concentrations were, without exception, lower than the leaf K^+ concentrations and both clones had similar K^+ concentrations in stems.

When plants treated with 300 mol m^{-3} NaCl, Na^+ and Cl^- concentrations were higher in the older leaves than in the younger leaves in both clones (Fig. 3). The oldest leaves (leaf 1) of the IL and SM plants contained 4300 and 220 mol m^{-3} Na^+ and Cl^- on a tissue water basis,

respectively, and the youngest leaves (leaf 5) contained 2500 and 400 mol m^{-3} Na^+ and Cl^- on a tissue water basis, respectively. The concentrations of Na^+ and Cl^- in the oldest leaf of the SM plants were 50% and in youngest leaf it was 20% of those in the IL plants while intermediate leaves of both IL and SM plants have concentrations between the two. In both clones the ionic concentration in roots were lower than stems which were lower than younger leaves and somewhat similar to young leaves of the SM plants but Na^+ and Cl^- in stems and K^+ in leaves of IL plants were higher than SM plants. In both clones, older leaves accumulated higher K^+ concentrations than the younger leaves, though mid-position leaves of the IL were exceptional, being loaded with higher K^+ concentrations.

The ionic concentrations on a tissue water basis for pooled shoots, all the leaves pooled and the stem of plants were also determined that indicate the contribution of leaves and stems to mean ionic concentration of shoots for plants cultured in non-saline medium for two weeks (Fig. 4). The leaves accumulated higher Na^+, Cl^- and K^+ concentrations than the stems so the pooled shoot ionic concentration lay between the two. The IL plants had higher Na^+ and Cl^- but lower K^+ concentrations than the SM plants and clone difference in ionic concentration were greater in leaves than in stems. At 200 mol m^{-3} NaCl level the Na^+ and Cl^- concentrations of the IL plants were always much higher than those of the SM plants which were similar to the above. High concentrations of Na^+ and Cl^- in the leaves of the IL plants were accentuated by the dehydration of the leaves while the K^+ concentrations of these leaves were also reduced. Same trend was among the plants cultured with 300 mol m^{-3} NaCl level that the Na^+ and Cl^- concentrations of the IL plants were always much higher than those of the SM plants but the older leaves of both clones were dehydrated severely, the Na^+ and Cl^- concentrations here were comparable with other salt levels applied.

DISCUSSION

From the results of the present study, it is clear that in the non-saline medium the SM clone of *A. stolonifera* accumulated higher Na^+ and Cl^- concentrations in various organs than the IL clone thus showing the slight tendency to behave like a halophyte in that it accumulated more ions from low salt medium than did the IL clone. Similar results have been reported in *Cynodon dactylon* and *Cenchrus ciliaris* (Akram *et al*., 2006). Under salt stress K^+ is in less concentration and its distribution within the plant is not related to the distribution of salt within the plant and this may be due that salt inhibits the K^+ uptake or induce an increase in potassium leakage from roots but however, the presence of Ca^{+2} may reduce K^+ loss and sodium uptake by plant roots (Nassery, 1979; Alian *et al*., 2000; Gurmani *et al*., 2006). Wainwright (1980) was of the view that there was less salt induced K^+ leakage from roots of the salt marsh clone than from the roots of the inland clone of *A. stolonifera*. In present study under salt stress stems had higher Na^+ and Cl^- levels than roots and Na^+ levels always remain higher than the Cl^- levels in both the organs but in leaves Na^+ and Cl^- levels showed an approximately stoichiometric relationship indicating that slightly more Na^+ has been taken up than Cl^- but according to Hussain *et al.* (2009) Na^+ and Cl^- levels in leaves were present in approximately equal concentrations at 3 dS/m in black seeds.

The gradual increase in concentration of NaCl in the rooting medium from 100 mol m^{-3} to 300 mol m^{-3} the SM plants showed consistency in their control of the distribution of Na^+ and Cl^- among their leaves. In both the clones the older leaves had higher Na^+ and Cl^- concentrations than the younger ones. Plant species form a continuum with respect to the accumulation of various ions in the individual organs (Ahmed *et al*., 1981; Gurmani *et al*., 2006). Many salt susceptible species maintain higher concentrations of Cl^- and Na^+ (Akram *et al*., 2006). Same is the case in SM plant showing better adaptation in salinity conditions due to inherent genetic variability in these plants. Such inherent genetic variabilities are the result of selection forces being reshaping the genomes of such plants under high salt stress to cope with the salinity stress. The distribution of Na^+ and Cl^- spread systematically up the plant with successively younger leaves taking an increasing share of the salt stress but low concentrations of Na^+ and Cl^- were maintained in the youngest leaves. This consistency of behavior may indicate that the same basic mechanisms are operating over the range of NaCl concentrations studied (Ashraf and O'Leary, 1996).

On the contrary IL plants did not show any consistency in their control over the distribution of Na^+ and Cl^- within the plant. But at 100mol m^{-3} and 200mol m^{-3} NaCl levels the IL and SM plants had a similar distribution of Na^+ and Cl^- in oldest and youngest leaves but IL plants had higher salt concentrations in the younger and intermediate age leaves were higher than those in the SM plants. At highest salinity level the pattern of distribution of Na^+ and Cl^- was again completely different in IL plants and it mainly due to the factor that IL plants have not been adapted to survive under higher salinity. At the peak salinity level the IL plants fail to retain the tendency to maintain the youngest and metabolically active leaves at low concentrations of Na^+ and Cl^- but SM plants still maintained this tendency even under considerable salt stress. Increase in the NaCl concentrations in the rooting medium cause a breakdown of the control over the Na^+ and Cl^- distribution at intermediate to low salinity and changing pattern of distribution of Na^+ and Cl^- within the plant organs reflect progressive damage to various physiological systems such as the water transport system and any control system

which may be present at the stem-leaf junction. At all salt levels the roots had the highest water content followed by stems and leaves. Younger leaves of both IL and SM plants had greater water content than the older ones. Salt had little effect on water content of leaves of SM clone but older leaves of IL clone were seriously affected.

The ionic concentrations on a tissue water basis are 1ikely to be the physiologically most important units of concentrations since ions exert their physiological effects in solution. The ionic concentrations reported here were expressed on a tissue water basis because comparisons between ionic concentrations of plant organs could be made more meaningful by expressing the results on a dry weight and on a tissue water basis. Small differences in water content per fresh weight bring about large changes in ionic concentrations on a tissue water basis. The leaves of salt treated plants had lower water content than those of control plants. They also had higher Na^+ and Cl^- concentrations on a dry weight basis. Consequently their leaves showed markedly higher ionic concentrations on a tissue water basis. This was particularly marked with salt treated inland plants. For example, in the 300 mol m^{-3} NaCI treated plants, on a dry weight basis, the youngest leaf (leaf 5) of the inland clone contained twice the concentration of Na^+ of the youngest leaf of the salt marsh clone. However, on a tissue water basis, the inland clone leaf contained 6.75 times the concentration of sodium of the salt marsh clone leaf.

REFERENCES

Ahmad, I., S.J. Wainwright and G.R. Stewart, 1981. The solute and water relations of *Agrostis stolonifera* ecotypes differing in their salt tolerance. New Phytol., 87: 615-629.

Akram, N.A., M. Shahbaz, H.R. Athar and M. Ashraf, 2006. Morpho-physiological responses of two differently adapted populations of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* (L.) to salt stress. Pak. J. Bot., 38(5): 1581-1588.

Alian, A., A. Altman and B. Heuer, 2000. Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci., 152: 59-65.

Ashraf, M., 2004. Some important physiological selection criteria for salt tolerance in plants. Flora, 1999. pp: 361-376.

Ashraf, M. and J.W.O. Leary, 1996. Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress: II. Water relation and photosynthetic capacity. Acta Bot. Neerl. 45: 29-39.

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

Cramer, G.R., G.J. Alberico and C. Schmidt, 1994. Salt tolerance is not associated with the sodium accumulation of two maize hybrids. Aust. J. Plant Physiol. 21: 675-692.

Flowers, T.J., 2004. Improving crop salt tolerance. J. Exp. Bot., 55: 307-319.

Grattan, S.R. and C.M. Grieve, 1999. Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic., 78: 127-157.

Gurmani, A.R., A. Bano and M. Salim, 2006. Effect of growth regulators on growth, yield and ions accumulation of rice (*Oryza sativa* L.) under salt stress. Pak. J. Bot., 38(5): 1415-1424.

Hussain, K., A. Majeed, K. Nawaz, K.H. Bhatti and M.F. Nisar, 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.

Munns, R., 2005. Gene and salt tolerance: bringing them together. New Phytol., 167: 645-663.

Munns, R., A.J. Richard and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57(5): 1025-1043.

Nassery, H., 1979. Salt induced loss of potassium from plant roots. New Phytol., 83: 23-27.

Niknam, S.R. and J. McComb, 2000. Salt tolerance screening of selected Australian woody species-a review. Forest Eco. Manage., 139: 1-19.

Rausch, T., M. Kirsch, R. low, A. Lehr, R. Viereck and A. Zhigang, 1996. Salt stress responses of higher plants: the role of proton pumps and Na^+/H^+ antiporters. J. Plant Physiol., 148: 425-433.

Serrano, R., J. Mulet, G. Rios, J. Marquez, I. de Larrinoa, M. Leube, I. Mendizabal, A. Pascual-Ahuir, M. Proft, R. Ros and C. Montesinos, 1999. A glimpse of the mechanisms of ion homeostasis during salt stress. J. Exp. Bot., 50: 1023-1036.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn. Lowa Stat University Press, Ames, Iowa.

Tester, M. and R. Davenport, 2003. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot., 91: 503-527.

Wainwright, S.J., 1980. Plants in relation to salinity. Adv. Bot. Res., 8: 221-261.

Wolf, B., 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Annal., 13: 1035-1059.