Growth Responses of *Plantago ovata* L. to Varying Levels of NaCl

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Abstract

The increasing human population demands more food, shelter and resources. However, it is not possible to bring arable lands under cultivation for aromatic and medicinal plants. The demand for medicinal drugs including ispaghol (*Plantago ovata*) is not only high but is also growing worldwide. The marginal, including salt-affected lands could be successfully utilized for the cultivation of non-conventional crops because these saline soils are not suitable for better vegetation cover due to poor physical properties and excess Na^+ concentration. Salinity being a global problem and a major constraint on food production worldwide affects plant growth and crop productivity and contributes to increased poverty in salt-affected areas. Among deleterious effects of high salt concentrations are ionic and osmotic imbalances, oxidative stress and also reduced photosynthetic capacity of plants. Assessing mechanism of salt tolerance in a crop species will ultimately help in devising a strategy to improve crop salt tolerance. The present study was conducted to see how and up to what extent varying degree of salinity stress affects the medicinally important non-conventional crop *Plantago ovata* (Ispaghol). From the results it is clear that increasing levels of NaCl in the growth medium reduced the growth of *P .ovata*. However, ANOVA revealed significant growth reduction at the highest salinity level (180 mM). The findings suggest that *Plantago ovata* might tolerate moderate levels of salinity. It can be tried for cultivation on marginal salted soils.

Keywords: salt tolerance; protein; amino acids; salinity; medicinal plants; abiotic stress

Khaliq, R., M. Zahoor, Z. U. Zafar and **H. R. Athar.** 2011. 'Growth responses of *Plantago ovata* L. to varying levels of NaCl'. *Iranian Journal of Plant Physiology* 1 (3), 157 - 167.

Introduction

The increasing human population over 8 billion will cause food insecurity for large population which demands more food, shelter and resources. According to an estimate improved crop productivity has resulted in increasing world food supplies and reducing the proportion of food insecurity for people living in developing countries (FAO, 2003). A number of factors decrease the crop productivity, of them, soil salinity is the most important factor and the main source of yield reduction (Boyer, 1982; Rehman et al., 2005; Munns and Tester, 2008; Reynolds and Tuberosa, 2008). In Pakistan, 6.8 million hectares out of 20.2 million hectares of cultivated land are affected with salinity (Khan, 1993). Salt-affected lands (both saline and alkali) don't support good vegetation cover while these lands could be beneficially utilized for the cultivation of aromatic and medicinal plants.

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Received: July, 2011
Accepted: September, 2011

High concentration of soluble salts affects plant growth, crop productivity and also the distribution of plants in arid and semi arid regions of the world. The adverse effects of soil salinity include ionic stress, osmotic stress, oxidative stress and nutritional disorders (Naidu and Rengasamy, 1993; Qadir and Schubert, 2002; Zhu, 2001). Two mechanism of salt tolerance, namely, 'salt exclusion' and tissue tolerance along with salt glands or bladders that excrete salt allow the plants to grow for long period of time in saline soil (Munns, 2002; Munns et al., 2006). Various strategies can be adopted to cope with salinity stress among which biological approach appears to be a long lasting, cheap solution and very effective in mitigating the problem of soil salinity world over (Ashraf, 1994; Epstein et al., 1980). Although various biological strategies for crop improvement against salt stress are being used, genetic improvement through screening and selection is the most effective strategy which depends on physiological or biochemical process. However, physiological parameters change with growth stage, thus a combination of these traits is used in screening for salt tolerance of the genotype (Noble and Rogers, 1992; Yeo et al., 1990). Plant growth regulators are widely applied to agricultural crops as a means of crops improvement by increasing stress tolerance of plants for salinity (Krishna, 2003). Moreover, plants have developed the complex antioxidant system to resist the salt stress (Hernández et al., 2000) and also responded to salt stress by osmotic adjustment (Jaleel et al., 2007). Research on agricultural, forage and fuel wood species has been carried out in relation to the effect of salinity but little work has been done on medicinal plants for exploring the possibility of using saline soils for their cultivation. *Plantago ovata* is a species of medicinal importance, generally grown in India, Pakistan & Iran. Its seeds contain 20 to 30% mucilage used by pharmaceutical companies in the treatment of chronic constipation, bowel cancer, amoebic dysentery, gastrointestinal irritation and it is very effective in reducing cholesterol level (Gupta et al., 1994). India is the main world-wide exporter of the seeds of *Plantago ovata* (Chaplin et al., 2000; Samad et al., 2002). The available literature includes the effects of NaCl salinity on the plant biomass, chlorophyll contents, total soluble proteins, total free amino acids and macronutrients. The findings might help increase the medicinal wealth of Pakistan by using its unproductive saline soils.

Materials and Methods

The experiment was conducted in glasshouse of Botanic Gardens of Bahauddin Zakariya University, Multan, Pakistan (30˚11N and 71˚28E). The average photoperiod was 8 h and day/night temperature was 26 ± 6˚C and 16± 4˚C during November-February 2009-10. The relative humidity ranged from 34.5 to 46.5 percent. Seeds of *Plantago ovata* were obtained from local market.

Ordinary river sand was washed thoroughly with tap water. The aluminum trays (200x100 cm) were filled with the sand which was 5 inches in thickness. The experiment was arranged in randomized complete block design with 4 salinity levels (0, 60, 120, and 180 mM NaCl). Salinity concentration was increased stepwise in aliquots of 60 mM to avoid the salt shock (Chartzoulakis and Loupassaki, 1997). Adequate amount of water was applied to each tray on alternate days to minimize evapo-transpiration loss. After 6 weeks growth, ispaghol plants were harvested. Plant roots were removed carefully from the sand and washed in tap water. Afterwards, the plants were separated into shoots and roots for determination of their fresh biomass and then blotted dry before recording their weights. Shoots and roots were oven-dried at 80˚C until constant dry weight and their dry weights were recorded. After 6 weeks growth of ispaghol plants, they were harvested. Before harvesting, chlorophyll contents (Witham et al., 1981), total soluble proteins (Bradford, 1976), total free amino acids (Hamilton and Van Slyke, 1943) were determined. For the analysis of macronutrients the youngest fully expanded leaf from each plant was sampled. The leaves of the comparable age were also sampled from different levels of NaCl for analysis of macronutrients. The macronutrients in plant shoots and roots were measured by the methods as described by Allen et al. (1986). Dry ground leaf (0.2 g) from each sample was digested in 2

mL of H_2SO_4-H_2O_2 digestion mixture until a clear and colorless solution was obtained. The volumes of the digested samples were made 55 mL with de-ionized water. K^+ in the shoots and roots was determined with a flame photometer (Jenway PFP7, Dunmow, Essex, UK). P was estimated by the method described by Jackson (1958) using a spectrophotometer (Hitachi U-2000, Tokyo, Japan) and N by Kjeldahl method (Allen et al., 1986).

The data obtained were subjected to a one-way ANOVA using the statistical computer package COSTAT (Cohort Software, Berkeley, USA) and means were compared with least significant difference following Snedecor and Cochran (1980).

Results

The result revealed that growth of *Plantago ovata* L. is strongly affected by all salt treatments. Increased salt concentration caused a decrease in growth. Strong reduction was observed mainly at the higher level of salt concentration (180 mM NaCl) compared to the

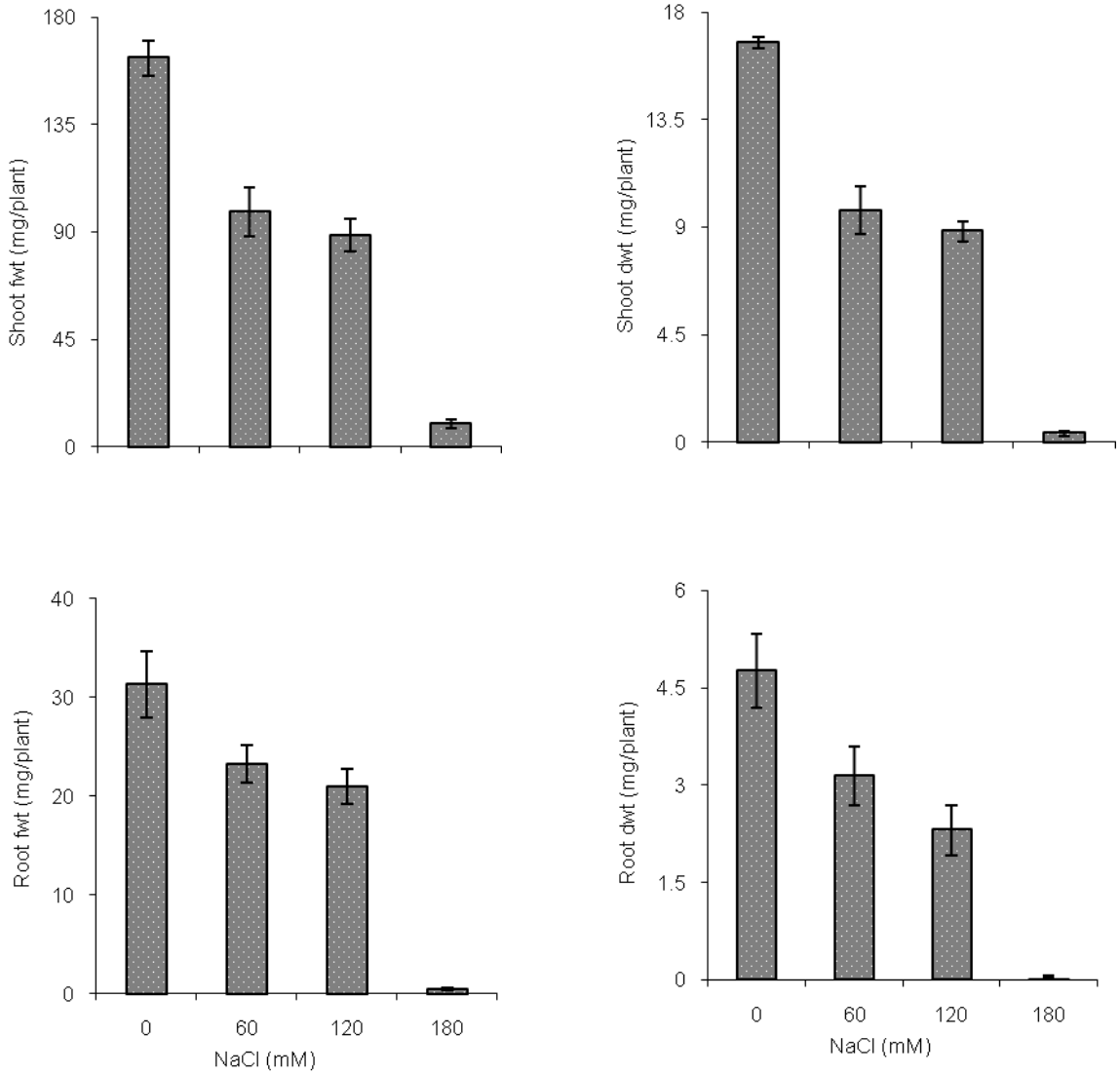

Fig. I. Growth attributes of *Plantago ovata* L.when two-week old plants were subjected to varying levels of NaCl stress for further four weeks.

plants in the control group (Fig. I).

Analysis of variance of data shows that addition of salt in the growth medium had significant (P≤ 0.001) reducing effects on fresh and dry weights of both shoots and roots (Table 1). Moreover, increasing levels of NaCl stress had caused a consistent decrease in the fresh and dry biomass of shoots and roots.

From the results, it is obvious that salt stress had slight inhibitory effect on chlorophyll

Table 1
Analysis of variance of data for growth attributes of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl salinity stress for further four weeks

SOV	**df**	Shoot fwt.	Shoot dwt.	Root fwt.	Root dwt.
Salt	3	4.3505***	0.0501***	0.2362***	0.0045***
Error	16	0.0748	0.0004	0.0051	0.0002
Total	19				

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level respectively; ns = non-significant

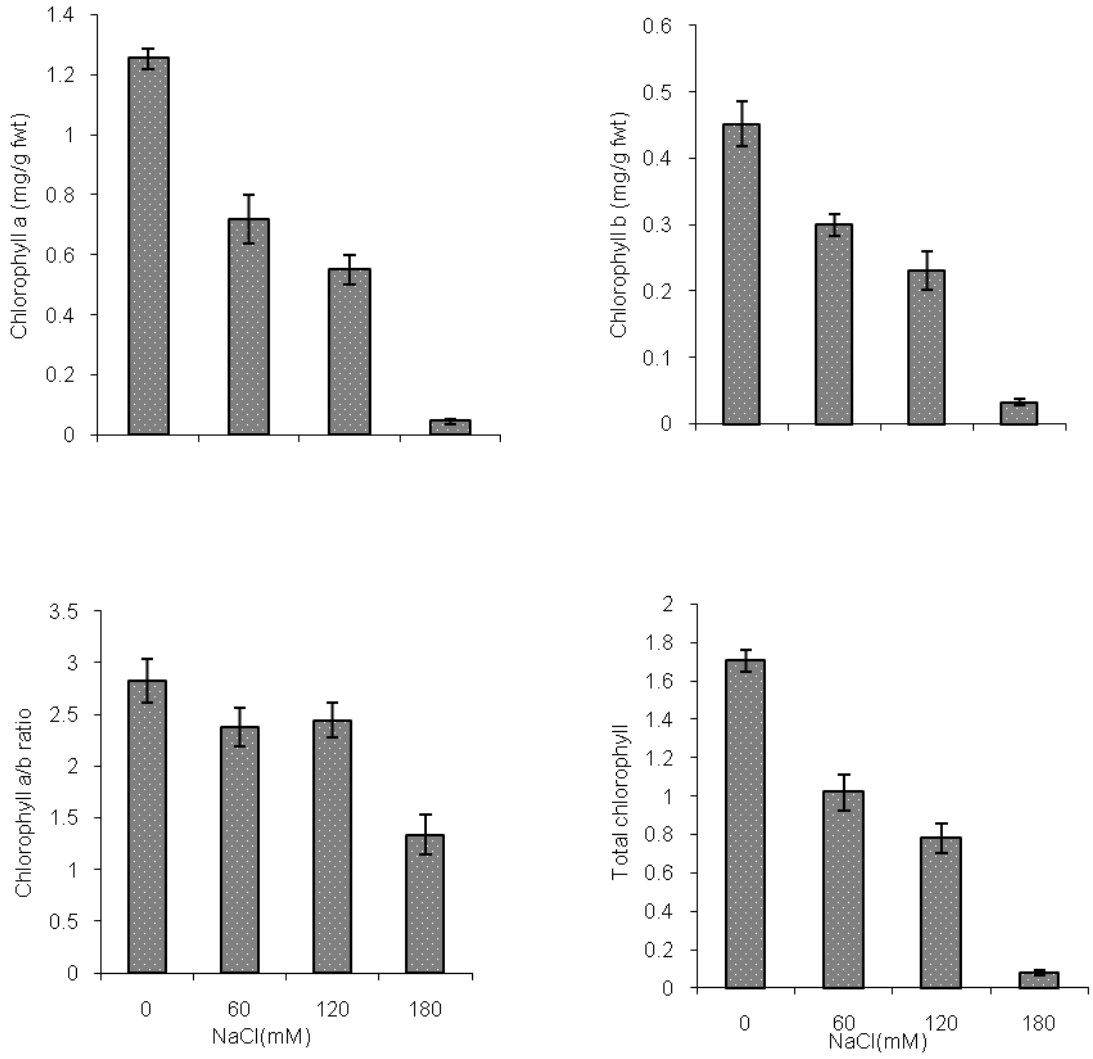

Fig. II. Chlorophyll contents of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl stress for further four weeks

'a' and total chlorophyll and significantly (P≤ 0.001) reduced chlorophyll 'b' (Table 2). Analysis of variance of data for chlorophyll contents of *P. ovata* L. is presented in Table 10. From the present study, it was concluded that the effects of various concentration of NaCl salinity were significant on the chlorophyll contents. It clearly showed that total chlorophyll concentration was low at high level of salt stress (180 mM) as compared to control level (0 mM) whereas it was higher in concentration from both chlorophyll 'a' and 'b' (Fig. II). Moreover, chlorophyll a/b ratio was enhanced at 180 mM NaCl salinity stress.

Total soluble proteins of *P. ovata* L.were slightly reduced by increasing levels of salt stress (Fig. III). At 0 and 60 mM NaCl there was no significant difference between total soluble proteins, whereas at 120 mM NaCl it decreased with increasing salt stress. Increasing salinity resulted in lower concentration of total soluble proteins. Statistical analysis also showed that there was highly significant reduction in total soluble proteins at all salt treatments (Table 3).

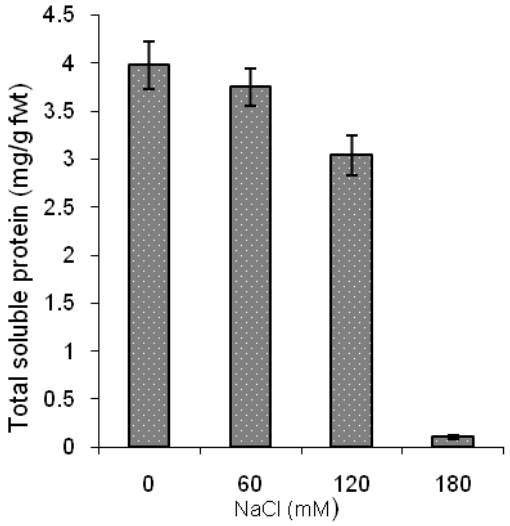

Fig. III. Total soluble proteins of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl stress for further four weeks

Total free amino acids were higher under saline conditions as compared to the non-saline conditions (Table 3; Fig. IV). ANOVA revealed that

Table 2
Analysis of variance of data for chlorophyll contents of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl salinity stress for further four weeks

SOV	**df**	Chlorophyll 'a'	Chlorophyll 'b'	Chlorophyll a/b	Total Chlorophyll
Salt	3	1.2395***	0.1506***	2.0293***	2.2504***
Error	16	0.0102	0.0023	0.1470	0.0181
Total	19				

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level respectively; ns = non-significant

Table 3
Analysis of variance of data for total free amino acids and total soluble proteins of *Plantago ovata L.* when two-week old plants were subjected to varying levels of NaCl salinity stress for further four weeks

SOV	**df**	Total Free Amino Acids	Total Soluble Proteins
Salt	3	22.2877***	15.9511***
Error	16	1.0358	0.1426
Total	19		

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level respectively; ns = non-significant

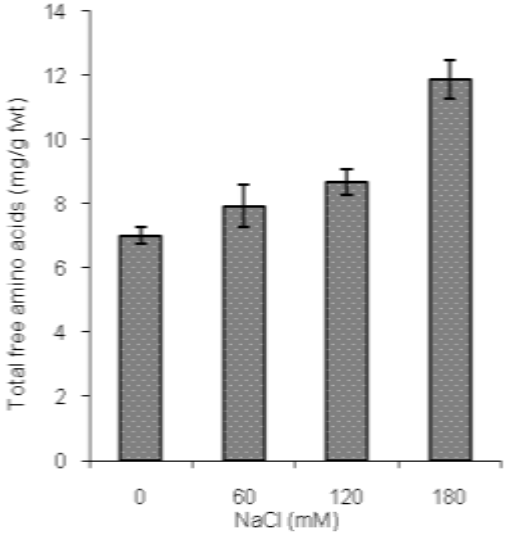

Fig. IV. Total free amino acids of *Plantago ovata* L. when two weeks old plants were subjected to varying levels of NaCl stress for further four weeks.

total free amino acids significantly (P≤ 0.001) increased with increasing levels of NaCl salinity. Moreover, total free amino acids were increased at high salinity level (180 mM NaCl) and reduced with decreasing the level of salt stress.

From the results it was concluded that all NaCl treatments had a significant effect on the macronutrients such as potassium and nitrogen in both leaves and roots of *P. ovata* L. (Table 4). Potassium (K) concentration was decreased in plant parts such as leaves and roots with an increase in salinity, whereas it was significantly (P≤ 0.001) enhanced in roots at both high and low levels of salt stress as compared to leaves.

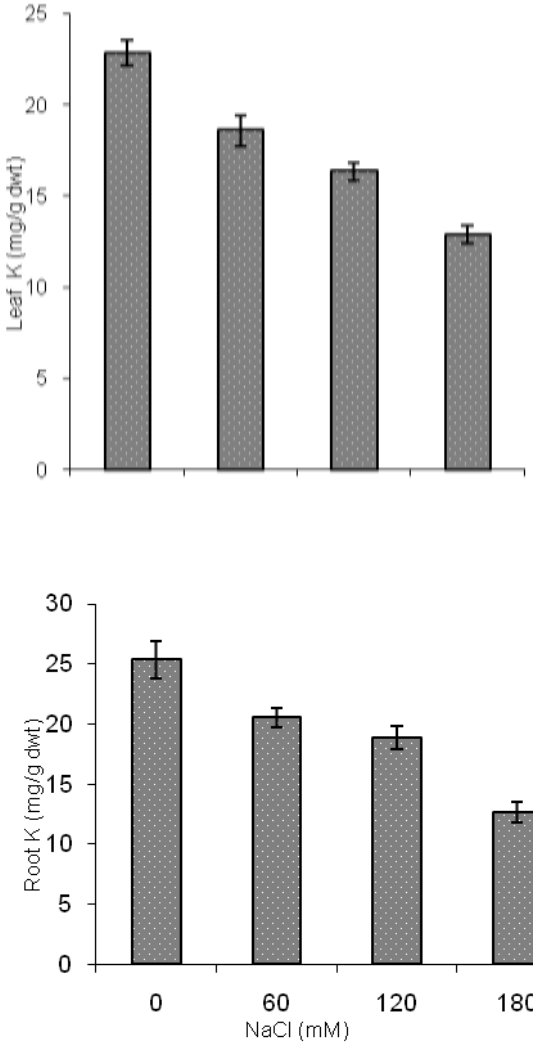

Fig. V. K concentration of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl stress for further four weeks

Table 4
Analysis of variance of data for macronutrients, K, P and N of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl salinity stress for further four weeks

SOV	**df**	Leaf N	Root N	Leaf K	Root K	Leaf P	Root P
Salt	3	127.491***	78.444***	87.364***	136.741***	0.0155^{ns}	0.0673^{ns}
Error	16	0.020	0.0641	1.748	4.552	11.779	2.508
Total	19						

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level respectively; ns = non-significant

Moreover, K^+ concentration was low at 60 and 120mM NaCl in both roots and leaves as compared to control but at higher salinity level both roots and leaves contained almost the same amount of potassium (Fig. V).

Phosphorous (P) concentration remained unaffected at all NaCl treatments. Both roots and leaves of *P. ovata* L. contained the same amount of phosphorous at varying levels of salt stress (Fig. VI). However, at all levels of salt stress phosphorous decreased non-significantly. Nitrogen (N) concentration in the leaves was higher as compared to the roots and in leaves. N concentration also decreased significantly ($P \leq 0.001$) with increase in salinity levels. Comparison of N concentration in the roots and leaves showed that roots had significantly lower concentration of N than leaves at various treatments of salt stress (Fig. VII).

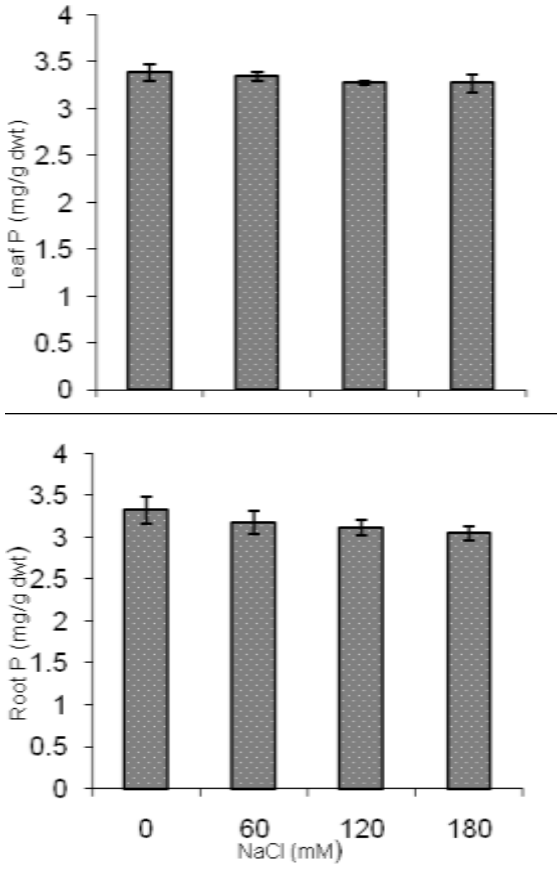

Fig. VI. P concentration of *Plantago ovata* L. when two-week old plants were subjected to varying levels of NaCl stress for further four weeks

Due to significant relation between salinity and growth parameters, it was easy to predict the relation between fresh shoot weight and dry shoot weight, chlorophyll contents, total soluble proteins and macronutrients, K and N. In this experiment, a slightly significant relationship was detected between the shoot fresh and dry biomass (Fig. I). But shoot fresh biomass slightly correlated with chlorophyll 'a' concentration. However, total soluble proteins significantly correlated with total chlorophyll. Moreover, total free amino acids negatively correlated with total soluble proteins. Significant relations were found between the root N and leaf K concentration.

Discussion

In the present study, it is evident from the results that fresh and dry shoot and root biomass of *P. ovata* L. consistently reduced with increasing level of salt stress. Salinity-induced growth reduction was also observed in many plant species, e.g., canola (Zadeh and Naeini, 2007), maize (Ashraf and McNeilly, 1990) and lucerne (Rogers et al., 2003). In the present results, depression of growth may be attributed to a decrease in water uptake from saline growth medium (Jamil et al., 2006; Flowers et al., 1990; Munns et al., 2006), reduced photosynthetic capacity, lower chlorophyll contents, low rate of cell enlargement (Arshad and Rashid, 2001; Redmann et al., 1994), oxidative stress and excessive accumulation of toxic ions such as Na and Cl in plant cells (Rogers and Noble, 1992; Shen et al., 1997; Flowers, 2004; Eraslan et al., 2007).

Dionisio-Sese and Tobita, (2000) also reported that high salt concentration reduced the plant growth either by increasing plant osmotic potential or specific ion toxicity. The reductions in the growth of ispaghol in the current study was noted by increasing salinity could also be the result of toxic effects of ions, which may lead to the suppression of uptake of other essential ions (Mer et al., 2000; Tester and Davenport, 2003; Flowers, 2004; Munns, 2005).

The root growth is the most important parameter for salt stress because root is the plant organ that has direct contact with growth medium to uptake all essential nutrients and

shoot supply it to the rest of the plant, thereby behavior of roots provides the useful information regarding the salt tolerance potential of the plants. In the present work, root growth was severely affected due to salinity. It is reported that root growth is sensitive to high salt concentrations as compared to shoot under saline conditions (Hajibagheri et al., 1989). Moreover, Ashraf et al. (2005) also reported that roots are rapidly reduced or prevented by salinity. Furthermore Zia and Khan (2002) also reported reduced growth under saline conditions in some other medicinal plants that also strengthen our findings.

From the results of the present study, it is clear that chlorophyll contents were reduced by applying the salt stress. The decrease in chlorophyll contents under saline conditions is also reported by Ashraf et al. (2005). The ANOVA revealed highly significant reduction in chlorophyll b as compared to chlorophyll a. This indicates that reduction in chlorophyll contents due to salinity may have been due to either slow synthesis or fast breakdown of chlorophyll pigments.

In the present study, our findings showed that increasing levels of salt stress seriously reduced the total soluble proteins. These results could be attributed to an increased proteolytic activity for the synthesis of osmoprotectants (Suarez et al., 2002). Statistical analysis of data showed that total free amino acids of *Plantago ovata* L. increased with increasing levels of salinity. From this perspective, it is clear that ispaghol with increased amino acids under salt stress conditions would have more advantages than the other plants that significantly reduce their amino acids by increasing salt levels. It was also reported that accumulation of organic compounds (total free amino acids) in the cytoplasm plays an important role in the osmotic balance of plants (Morgan, 1992).

Plants need essential macronutrients such as K, P and N for their growth and development. It was investigated that potassium enhances the synthesis of carbohydrates and also increases cell wall thickness and is also essential for plant survival in saline habitats thereby contributing to osmotic adjustment and overall water balance of plants.

From our findings, it was clear that potassium (K) concentration was decreased in plant parts such as leaves and roots with increased salinity. For this reason, high salt uptake hindered the uptake of other nutrient ions, especially potassium, leading to K^+ deficiency. In another study on maize hybrids, it was reported that reduction in K^+ contents was due to presence of excessive Na^+ in growth medium which had an antagonistic effect on K uptake in plants (Khan and Ashraf, 1992; Ashraf et al., 2005; Akram et al., 2007).

Phosphorous (P) concentration remained unaffected at all NaCl treatments. Both roots and leaves of *P. ovata* L. were contained the same amount of phosphorous at varying levels of salt stress.

Nitrogen (N) concentration in the leaves was higher as compared to the roots whereas in leaves, N concentration decreased significantly ($P \leq 0.001$) with increase in salinity levels. Nitrogen plays a vital role in chlorophyll biosynthesis, protein synthesis and photosynthesis (Walker and Weinstein, 1991; Ashraf and Rehman, 1999) because photosynthesis depends on leaf chlorophyll contents as it linearly correlates with leaf nitrogen (Dingkuhn et al., 1992).

However, it is clear from the results that N and K accumulation reduction in both roots and leaves under the salt stress were directly related to their growth. Anwar et al., (2001) observed that N and K accumulation decreases in plant parts with increased salinity and protein biosynthesis generally declines under the salt stress which strongly supports our findings.

Research on the agricultural species has been carried out in relation to salinity but little work has been done on economically important medicinal plant, ispaahol for exploring the possibility of using salted soils for their cultivation. Our results indicated that the cultivation of medicinal plants like *Plantago ovata* L. in saline area may be possible because it is clearly a salt tolerant crop of winter season and will provide not only economic returns but also improve these unproductive soils.

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