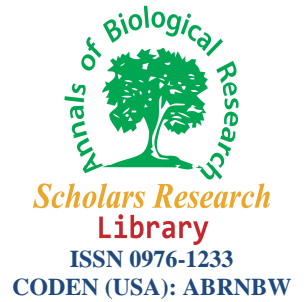




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The effect of NaCl on the growth and Na⁺ and K⁺ content of barley (*Hordeum vulgare*, L.) cultivares

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ABSTRACT

In order to investigate the effect of salt stress on growth and sodium and potassium ion content of barley (*Hordeum vulgare*, L.) plants, four cultivars were subjected to salinity levels (control, 50 and 100 Mm) in hydroponics. Salt stress was imposed to cultivars in root establishment stage (4 leaves). Results showed that under stress condition, growth of seedlings decreased dramatically. Ion content of barley cultivars changed in salinity conditions. Na⁺ concentration were increased with increasing NaCl levels, whereas K⁺ concentration and K⁺/Na⁺ ratio were decrease with rising of the NaCl level. Significant differences were observed between barley cultivars for various salt tolerance-associated traits under salinity stress. The results clearly showed that Lisivy cultivars had the highest salt tolerance compared with the other three.

Keywords: salinity stress, Barley, ion content, salt tolerance

INTRODUCTION

One of the most important effective factors on plant growth is salt stress (Satorre, 1999). Salinity affected about 7% of the earth lands (about 930 million hectares) and it is outspreading (Barsa & Barsa 1997). Soil salinity is a considerable problem adversely affecting physiological and metabolic processes, finally diminishing growth and yield (Ashraf and Harris, 2004). The constituent cautions of total soluble salts in soils are usually sodium (Na⁺), calcium (Ca²⁺), and magnesium (Mg²⁺) and the anions are chloride (Cl⁻), sulphate (SO₄²⁻) and carbonate. However, Na⁺ dominates the cautions and Cl⁻ the anions in the majority of saline soils to the extent that NaCl comprises from 50–80% of the total soluble salts (Rengasamy, 2010).

Barley (*Hordeum vulgare* L.) is grown as a commercial crop in one hundred countries and is one of the most important cereal crops in the world. Barley assumes the fourth position in total cereal production in the world after wheat, rice, and maize (FAO, 2004). Generally, barley is considered as salinity tolerant crop. It has been particularly satisfactory as one of the

Early crops planted in the process of reclamation saline soils (Ayers and Westcot, 1985). The indication of good salinity tolerance at one growth stage such as germination and seedling does not necessarily mean that other stages will also have good salt tolerance. Yield components and growth parameters also show differential responses to salinity stress. Ayers et al. (1952) found that in barley and wheat seed production was decreased less than shoot dry weight by salinity. Likewise, at low salinities root growth is often less affected, or sometimes even stimulated by salinity, compared to shoot growth. In muskmelons, salt tolerance decreased in the following order: total vegetative dry weight > total vine yield > fruit yield > marketable yield (Shannon and Francois, 1978).

Salt sensitivity in some crops has been attributed to the failure of plants to keep Na^+ and Cl^- out of the transpiration stream and, consequently, the cytoplasm of the shoot tissues (Flowers et al, 1977; Harvey, 1985). Under salt stress a plant must absorb nutrients and restrict the uptake of toxic ions at lower water potentials than usual. Munns and Termaat (1986) divided salt stress into short- and long-term effects. Short-term effects occur in a matter of days and involve decreased shoot growth, possibly as a result of the root response to water deficit. Long-term effects occur over weeks and result in maximum salt loads in fully expanded leaves and a reduction in photosynthetic activity. Flowers and Yeo (1986) noted that salt damage in leaves of sensitive species may be the result of excess apoplastic ion concentrations or ion toxicity effects on metabolic processes in the symplast.

For most plants to tolerate salinity, Na^+ and Cl^- uptake must be restricted while maintaining the uptake of macronutrients such as K^+ , NO_3^- and Ca^{2+} . The mechanisms of Na^+ and K^+ transport in plants under salt stress have been extensively researched and reviewed (Amtmann and Sanders, 1998; Shabala and Cuin, 2008). Reduced Na^+ loading into the xylem is one of the main mechanisms of salinity tolerance and it is often considered one of the most crucial features of restricting Na^+ accumulation in plant tissues (Tester and Davenport, 2003; Munns and Tester, 2008). Among the crop plants, an extensive research has been conducted on the effect of salinity on barley. (Cramer and Nowak, 1992) This crop species is salt tolerant (Mer et al, 2000; Brady and Weil, 1996) Researchers found that differences in the salt tolerance of barley was related to their ability to regulate ion transport (Greenway, 1963; Greenway et al, 1965). In particular, Na^+ exclusion seemed to be important (Greenway, 1962). Munns et al (1982) assessed the contribution of osmotic effects of salinity relative to its ionic effects and concluded that inhibitory effects on growth were mostly due to osmotic and not ion specific effects.

MATERIALS AND METHODS

This experiment was conducted in Biology Department, Faculty of Science, and University of Mohaghegh Ardabili, Iran.

The seeds of four barley cultivars (*Hordeum vulgare*, L.), Dasht, Lisivy, Sahra and Sahand were obtained from seed and plant Improvement Institute, Karaj, Iran.

The experimental design was a factorial completely randomized design comprising three treatments \times four barley cultivars with three replicates.

A solution culture experiment was conducted to assess the effect of different concentrations of NaCl on the growth of genotypes. Three concentrations (0, 50 and 100 Mm) of NaCl Hoagland's solutions were prepared by dissolving a mixture of NaCl salt in nutrient solution. All treatment solutions had a background of modified Hoagland's solution for nutrient supply, the composition of which (in mM) was: NH_4NO_3 (0.2), KNO_3 (5), $\text{Ca}(\text{NO}_3)_2$ (2), MgSO_4 (2), KH_2PO_4 (0.1), NaFe (III)-hydroxyethyl ethylenediamine triacetic acid (HEDTA) (0.05), H_3BO_3 (0.01), MnCl_2 (0.005), ZnSO_4 (0.005), CuSO_4 (0.0005), and Na_2MoO_3 (0.0001). The experiment was operated in greenhouse with day/night temperatures of approximately 25/18°C.

At 12–15 d after germination, when the fourth leaf was beginning to appear, the NaCl treatments were introduced over 10 d (Genc et al, 2010). Plants were harvested after 24 d, seedlings were pulled from pots and then shoot heights were measured. Roots and shoots of seedlings were cut and dried in oven (68°C) for 48h and then dry weight of roots and shoots were measured separately.

For Measurements of Na^+ and K^+ concentrations, the leaves were dried in 60°C for 48 h. Then 1 gr of leaves was powdered and burned in 560°C to obtain ash then ashes digested in 10 ml of 1N HCL. The concentration of Na^+ and K^+ in the digested samples was determined using a flame photometer (Model 420, Sherwood, Cambridge, UK)

Statistical analysis: Data were analyzed using ANOVA to determine if significant differences were present among means. Differences among the mean values were assessed by Duncan test using the SPSS program. Diagrams designed by Excel software.

RESULTS

Effect of salt stress on barley growth

When grown hydroponically, barley cultivars showed differential responses to salt. The growth of all barley cultivars was inhibited by NaCl even at low concentration (50 mM) in the root Medium. Shoot heights of all cultivars were reduced significantly with increasing in NaCl levels (Fig1a). In Dasht and Lisivy, shoot heights were reduced by 6% in NaCl treatment, whereas, shoot height was decreased in Sahra by 18%. The highest decrease of

shoot height due to NaCl treatment was observed in Sahand (23%). Increased levels of salts in the solution reduced shoot dry weights of all the varieties but the responses of genotypes differed (Fig 1b). There was not significant different in shoot dry weight between saline solution and control for Lisivy. In Dasht and Sahra shoot dry weight reduced by 14% and 37%. Sahand had the highest decrease of shoot dry weight under NaCl treatment compared with the other three (43%). Reduced growth under saline conditions is a common response of many plant species including barley (Mahmood et al., 1996). Garthwaite et al. (2005) reported that among *Hordeum spp.*, growth of *H. vulgare* was more adversely affected by salinity compared to wild species. In other studies; growth of barley seedlings was inhibited at 150 mM NaCl (Cramer et al., 1989).

Salt stress had a negative effect on root growth and caused reduction of root dry weight in cultivars (Fig 1c). In Dasht and Sahra the root dry weight was reduced by 8% in NaCl treatment. Whereas, the root dry weight of Lisivy was reduced by 14%. The highest decrease of root dry weight due to NaCl treatment was observed in Sahand and it decreased by 39%. Root is the first organ of plant that expose to salinity. Decrease of root dry weight is due to ion toxicity, imbalance of nutritional elements and disorder of osmotic regulation (Tester & Davenport, 2003).

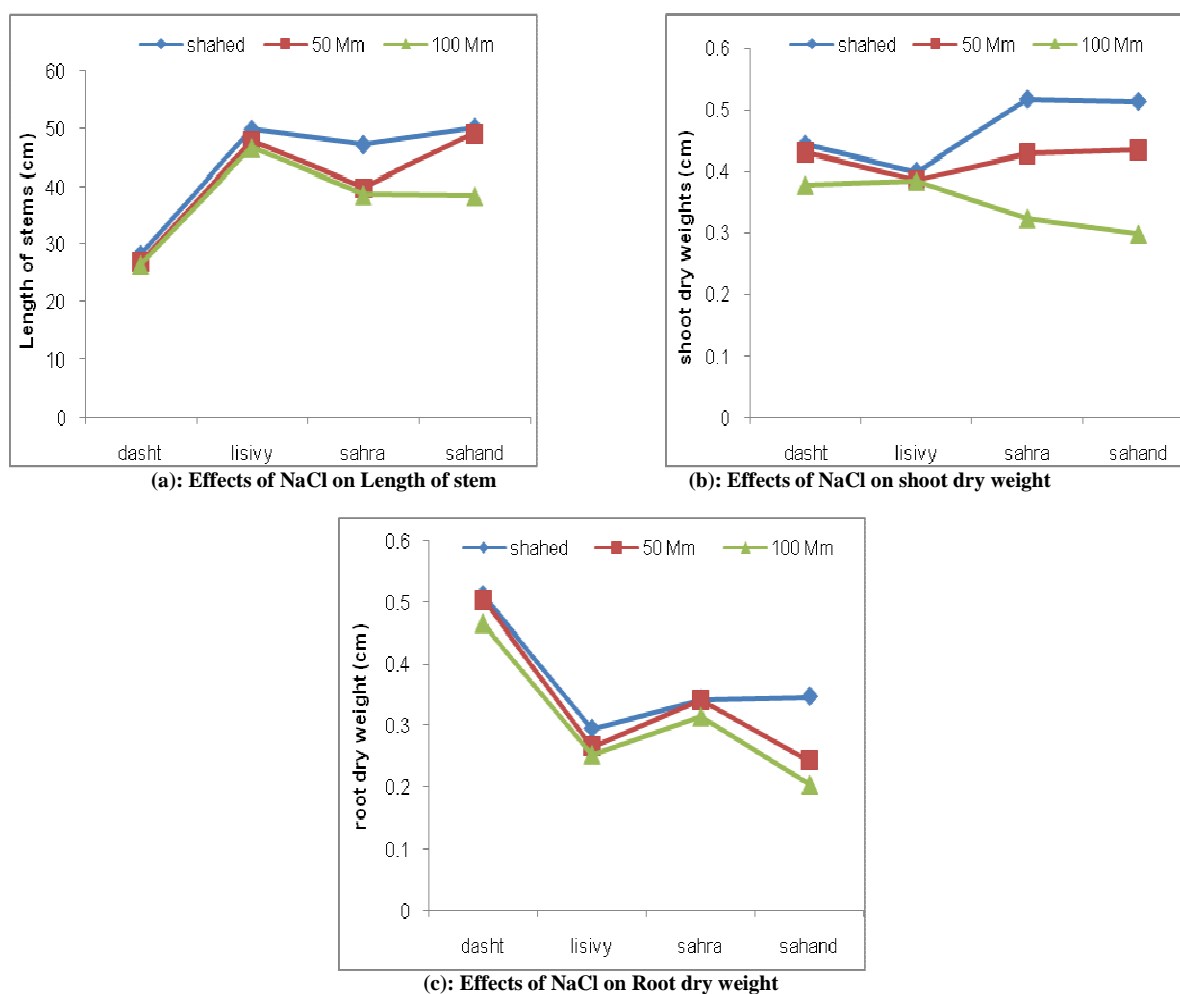


Fig 1: shoot height and shoot and root Dry weight measurements of barley plants grown hydroponically in control (1 mM) and salt-treated (50 and 100 mM NaCl) conditions.

Effect of salt stress on ion content

Plant responses to NaCl were determined by measuring Na^+ and K^+ concentrations in the leaf by flame photometry following exposure to salt for 10 day. Fig 2(a) shows that the concentration of Na^+ in leaves was much higher in plants grown under salt stress, regardless of variety. After salt treatment, there was a 99% increase in Na^+ level in Sahand. Sodium concentration increased in Dasht and Sahra by 31% and 73%, respectively. Lisivy had the lowest increase of Na^+ concentration under NaCl treatment compared with the other three (15%). Chen et al (2005) reported that sodium concentration of root and shoot increased and K^+/Na^+ ratio was decreased under salinity stress. Increase of sodium uptake and reduction of sodium itrance to vacuole cause increase of sodium concentration in apoplast. Potassium concentrations in all cultivars decreased with increasing NaCl in treatment solutions (Fig 2(b)). There

was a similar 16% decrease in K^+ levels after 10 day in Dasht and Lisivy following exposure to salt. Decrease of K^+ concentration in Sahra was 25%. Sahand consistently maintained highest decrease in concentration of K^+ (36%). Thus causing decrease in K^+/Na^+ ratio (Fig 2(c)). Sahand have highest decrease of K^+/Na^+ ratio (4/2%), whereas this ratio fell *above* 4/2% for the other cultivars, with Lisivy having the highest ratio.

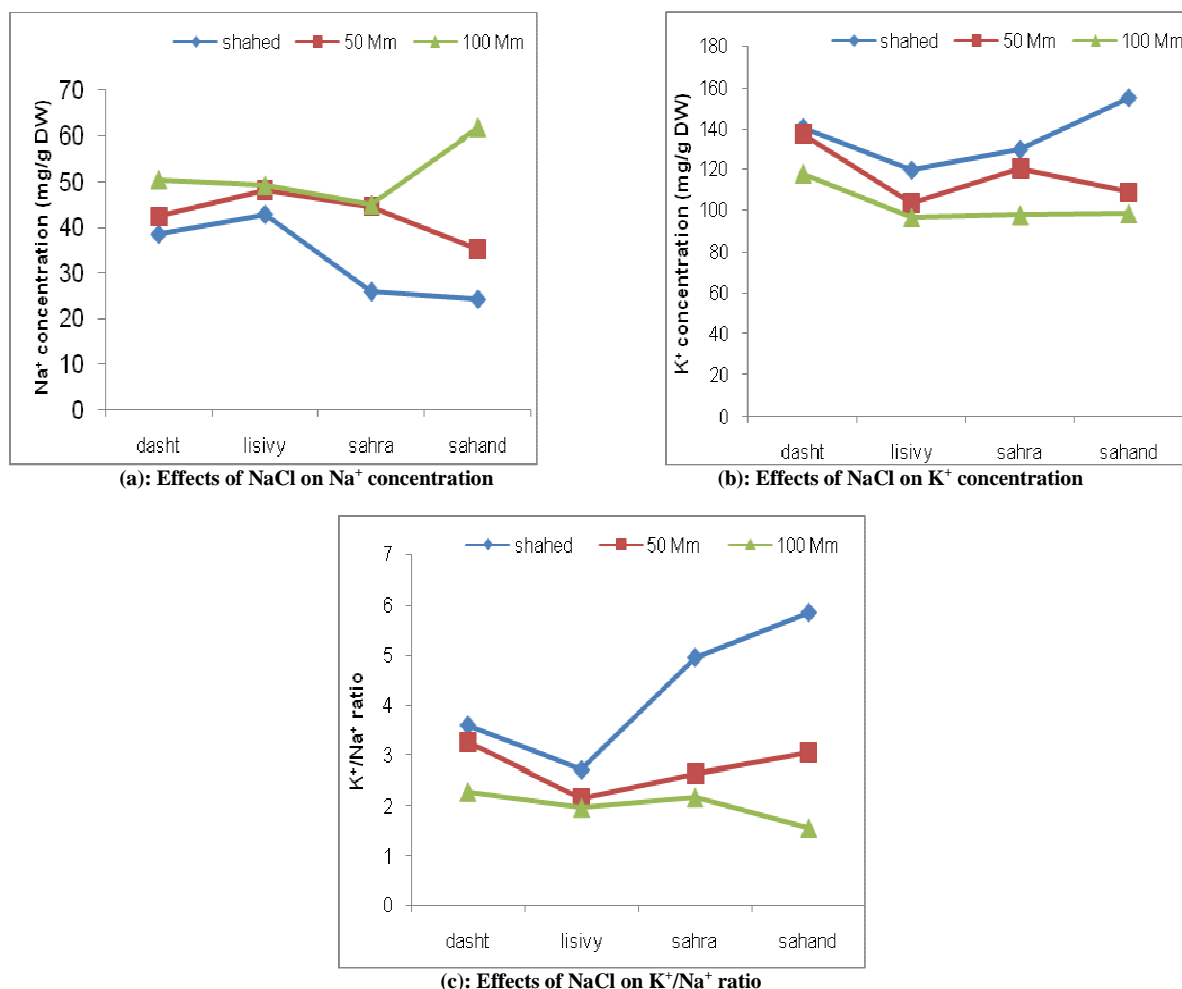


Fig 2: Na^+ concentration, K^+ concentration and K^+/Na^+ ratio measurements of barley plants grown hydroponically in control (1 mM) and salt-treated (50 and 100 mM NaCl) conditions.

DISCUSSION

Four barley cultivars grown hydroponically under conditions of high Salinity, showed very differently over the 10 day period. The results clearly showed that increases in salinity decreased growth of barley plants. However, plant species differ in their sensitivity or tolerance to salts. Sahand was severely affected, having a growth reduction of up to 23% of shoot height. In addition, dry weight of shoot and root decreased by 43% and 39%, respectively. Different responses were seen in barley cultivars after 10 day of salt treatment: Sahand essentially ceased growing, whereas Lisivy resumed growing at rates similar to those of untreated plants (Fig. 1). Previous studies have demonstrated retardation of germination and growth of seedlings at high salinity (Grog and Gupta, 1997; Ayers and Hayward, 1948). The depressive effect of salt on the growth is, according to Hajji *et al.* (1999), the results of a reduction in the osmotic potential of the soil solution around the roots, an increase in the accumulation of some ions in harmful concentrations in tissues and a modification of the nutritional statute of the essential ions to the growth and the development. In other studies, it was shown that the growth of aerial organ was inhibited under salt stress by the decrease of root growth (Cramer *et al.*, 1989; Yeo *et al.*, 1991; Rengel, 1992). According to Levigneron *et al* (1995), the increase of soil salinity is translated by an immediate reduction of shoot growth.

In this study, the NaCl Hoagland's solutions were designed to give concentrations of the Na^+ and k^+ ions (Fig 2). Using barley cultivars with known genetic variation in salinity tolerance and in Na^+ and k^+ uptake also assisted in

distinguishing the toxic effects of Na⁺ ions. The concentrations of Na⁺ increased in barley plants exposed to salt stress. Sahand had the high concentration of Na⁺ in salinity condition (up to 99%), whereas it was increased in Lisivy only up to 15%. Na⁺ toxicity is strongly linked to plant's ability to maintain uptake and within plant distribution of K⁺ (Kader & Lindberg, 2005).

There was a marked difference in the K⁺ concentration in barley cultivars leaves following salt stress. NaCl treatment caused decrease in potassium concentration. Thus causing decrease in K⁺/Na⁺ ratio (Fig 2). In the present studies, a similar trend was observed as indicated by lower K⁺/Na⁺ ratios in plant leaves in 100 mM NaCl treatment. In Dasht and Lisivy leaves after 10 day of salt stress, potassium concentration decreased by 16% and these has highest K⁺/Na⁺ ratio compare with the other cultivars. Maintenance of high K⁺ concentrations in salt-tolerant cultivars may be one of the mechanisms underlying their superior salt tolerance (Maathuis and Amtmann, 1999; Britto et al., 2010; Tester and Davenport, 2003).

Selective K⁺ uptake has been reported to be associated with salt tolerance in many species (Mahmood et al., 1996). However, higher K⁺/Na⁺ ratio does not always correlate with salt tolerance. No K⁺/Na⁺ selectivity occurred in sugar beet (Hasegawa & Yoneyama, 1995), a salt tolerant species whereas *Sesbania rostrata* having medium salt tolerance exhibited high discrimination for K⁺ uptake (Mahmood, 1998). In the present studies, clear differences were noted among the cultivars for K⁺ uptake and within plant distribution under NaCl treatment (Fig 2). K⁺/Na⁺ ratios in leaves of more salt tolerant cultivars (Lisivy and Dasht) were higher than those in the less tolerant cultivars. Further, selective transport of K⁺ from root to shoot was more efficient in these cultivars as indicated from higher K⁺/Na⁺ ratios in leaves. In this experiment, growth response was related to the efficiency of cultivars to maintain K⁺ uptake under stress conditions.

CONCLUSION

In general this study indicates that salinity led to a significant decrease in the growth parameter as shoot height, root and shoot dry weight of all 4 barley cultivars. The result also pointed out clearly that salinity changed ion uptake in root. Salinity caused increase in Na⁺ concentration, Increase in Na⁺ uptake inhibited K⁺ uptake and decreased K⁺ concentration and K⁺/Na⁺ ratio. Also, our results showed that Lisivy and Dasht cultivars have the highest tolerance while Sahand has the lowest tolerance under the same condition of salt stress.

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