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Effects of Salt Stress on Vegetative Growth and Ion Accumulation of Two alfalfa (*Medicago sativa* L.) Cultivars

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Abstract

Salt stress is one of the most chalanging abiotic stresses affecting natural productivity and causing significant crop losses worldwide. A greenhouse experiment was conducted to evaluate the response of two alfalfa cultivars (Bami and Hamedani) to 6 levels of salinity (0 as control, 25, 50, 75, 100, 125 mM NaCl) at the College of Agriculture, Shiraz University, Shiraz, Iran in 2008. Plant dry weight per pot in both cultivars decreased with increasing salinity levels. However, Bami as compared to Hamedani, with the lowest Na^+ sequestration, produced the greater dry matter weight. Leaf area per pot was significantly affected by salt stress with Bami cultivar showing a higher leaf area than Hamedani. Na^+ accumulation also increased by increase in the salinity level in either one of the cultivars; however, Na^+ sequestration of Bami as compared to Hamedani, was lower due to Na^+ exclusion mechanisms occurring in this cultivar. Although Cl^- accumulation increased with increasing salinity level in either one of the cultivars, Cl^- accumulation was higher in Hamedani than in the other cultivar. Similar to K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio also decreased by an increase in salt stress levels and there were highly significant differences observed between 25 and 125 mM of NaCl in either one of the cultivars. There was a strong positive relationship observed between plant dry matter weight and leaf relative water content for both Bami (R^2=0.94) and Hamedani (R^2=0.96) cultivars under salt stress conditions. All in all, it appears that less adverse effect of salinity on Bami cultivar has made it suitable for growth in saline soils as compared to Hamedani in saline areas prevailent in south Iran.

Keywords: Alfalfa; NaCl; K^+/Na^+ ratio; Dry weight

1. Introduction

Soil salinization is a seriously challenging problem in the entire world. It has substantially extended, causing great losses in crop productivity. It is estimated that about 954 million hectares of land around the world are already salinized (Da Silva and Nogueira 2008). According to Munns *et al* (2006), plant growth response to salinity involves two phases. In the first phase, the presence of salt in the soil solution reduces the ability of the plant to take up water, leading to slower growth. This is the osmotic or water- deficit effect of salinity. The second phase results from the toxic effects of salt inside the plant. This is the salt specific or ion-excess effect of salinity. In some plants, these phases may occur sequentially (Munns *et al* 2006).

Na^+ is a harmful ion responsible for a majority of agricultural losses whereas K^+ is an essential ion to plant growth. (Taiz and Ziger, 2002). Under a typical NaCl-dominated salt environment in nature, accumulation of high Na^+ in the cytosol, and thus high Na^+/K^+ ratios, disrupts enzymatic functions that are normally activated by K^+ in cells (Munns *et al* 2006).

Salt tolerance is usually assessed as the percent dry matter weight production in saline versus non-saline conditions over a prolonged period of time (Sadeghi and Emam, 2006).

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Dramatic differences in response to salinity are found among plant species. For example, after some time in 200 mM NaCl a salt-tolerant species such as sugarbeet might have a reduction of only 20% in dry weight, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might end up with total death (Munns 2002).

Salt affected lands are generally less productive and less profitable especially if salt-sensitive (and often more valuable) crops such as alfalfa, can not be grown on them (Suyama *et al* 2007). In legumes, salt stress from 50 to 200 mM NaCl significantly limits productivity by interfering with plant growth (Cordovilla 1999). Salt tolerant alfalfa cultivars such as Salado grew well, with forage quality very high, but only under irrigation and on non-saline soil or soil salinity of less than 7dS/m (Suyama *et al* 2007).

In most southern provinces of Iran, salinity is an ever growing, problem, particularly in irrigated agricultural areas with rising water tables, poor water quality and inadequate soil drainage (Sadeghi and Emam 2006). The aim of this research was to estimate the vegetative growth, as well as salt ions accumulation in two varieties of alfalfa (Bami and Hamedani) under salt stress in controlled conditions.

2. Materials and methods

To evaluate the effects of sodium chloride on growth and biomass production of two alfalfa cultivars (Bami and Hamedani) under 6 levels of salinity namely: 0 as control, 25, 50, 75, 100, and 125 mM NaCl a greenhouse experiment was designed and conducted. The experiment was carried out in a greenhouse at the College of Agriculture, Shiraz University, (52° 46' E, 29° 50' N) 12 km north of Shiraz, Iran, on a Fine mixed, mesic Typic Calcixerpets soil. The air temperature was in the range of about 25 to 30° C, with 70 to 80% relative humidity, and light intensity in the range of about 600-1000 µmol m^{-2} s^{-1}. A randomized complete design with four replications was employed. Soil properties are shown in Table 1.

Table 1. Soil properties (0-30 cm) before sowing

Soil texture	Sand (%)	Silt (%)	Clay (%)	OC (%)	pH	EC (dS/m)	Total N (%)	P (mg kg^{-1})	K (mg kg^{-1})
Silty loam	7	67.6	25.4	0.91	7.1	0.05	0.09	16.1	463

Pregerminated seeds were sown in 5 kg plastic pots filled with fertilized soil of 20, 25 and 10 N, P, K mg kg^{-1}, respectively. Fifty seeds of each cultivar were sown in each pot, on 28th April 2008, thinned to twenty seedlings at two-leaf stage, and harvested at 10% flowering stage on 3 July, 2008. The plants were salt treated, after they were at their two-leaf stage.

Total plant leaf area per pot was assessed at harvest bye use of a leaf area meter (Model 3000, LI-COR Inc.: Lincoln, NE) 67 days past salt treatment. At final harvest, the forage was cut, rinsed briefly in deoinized water to remove on surface accumulated salts and dust, weighted and dried in an oven of 60°C heat for 48 hours, to determine the dry matter content. The dried tissues were ground (to pass a 1 mm mesh) in a Thomas-Wiley laboratory mill for Na^+ and K^+ and Ca^{2+} quantification. The contents of Na^+, K^+ and Ca^{2+} in the mineral extract were then determined using a flame photometer (Model Jenway PFP-7) (Jameel Al-Kahayri, 2002). Chloride was determined by titration following the samples being oven-dried and then the ground material being put to extraction in demineralized water for 30 min (Chen et al 2001). The percentage leaf Relative Water Content (RWC%) was calculated according to Beadle *et al* (1993) using the equation:

RWC (%)= [(FW - DW)-(TW-DW)].100

where FW is the fresh weight, DW the dry weight and TW the turgid weight. The data were subjected to analysis of variance, and the means separated using Duncan's Multiple Range Tests ($p \leq 0.05$) through SAS, 2000.

3. Results and Discussion

3.1. Effect of salt stress on vegetative growth of alfalfa

Dry weight per pot for Bami cultivar was significantly decreased by increase in salinity level from 75 to 125 mM NaCl while for Hamedani cultivar this decrease was found when salinity level increased from 50 to 125 mM of NaCl (Fig. 1). Therefore, Bami cultivar with a lower Na^+ accumulation produced the greater dry weight as compared to Hamedani (Fig. 1 & Table2). Suyama *et al* (2007) has reported that under salt stress conditions of 100 and 125 mM NaCl, a respective decline of 50 and 75% in dry matter production occurred in alfalfa (cultivar 801S). Evidently the lower dry matter production was observed at high salinity level treatments. This is probably why long term irrigation of Salado alfalfa with water of higher than 75 mM of NaCl salinity should not be recommended (Banuelos *et al* 2003). Hashemi and Hajrasoliha (2001) reported that at 200 and 250 mM of NaCl the plants died because of salt accumulation followed by toxic effect on alfalfa.

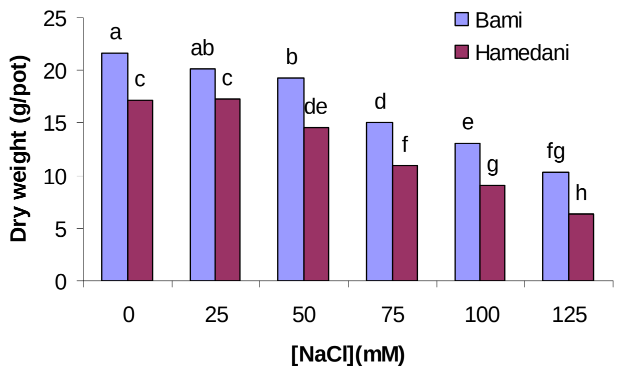

Fig. 1. Effect of NaCl concentration on dry weight per pot of two cultivars of alfalfa. Means followed by similar letters are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

Table 2. Effect of salinity on chemical composition of two cultivars of alfalfa

Cultivars	Salinity (mM)	K^+ (mmol Kg^{-1} dry weight)	Na^+(mmol Kg^{-1} dry weight)	Ca^{2+}(mmol Kg^{-1} dry weight)	Cl^- (mmol Kg^{-1} dry weight)	K^+/Na^+	Ca^{2+}/Na^+
Bami	0	606a	220j	290a	178i	2.75a	1.31a
	25	605a	227j	289a	180i	2.76a	1.33a
	50	600a	240i	285b	182i	2.50b	1.01d
	75	456b	305f	161d	381f	1.49d	0.52e
	100	311c	420e	140e	420d	0.74e	0.25g
	125	211d	565c	102g	503c	0.37f	0.16h
Hamedani	0	583a	238i	278c	192h	2.45b	1.15b
	25	582a	242i	273c	195h	2.40b	1.13b
	50	541a	251h	270c	298g	2.16c	1.08c
	75	416b	413e	158d	390e	1.01e	0.38f
	100	208d	571c	131f	531b	0.36f	0.22g
	125	150d	643a	96h	622a	0.23f	0.16h

Means at each column followed by similar letters are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

Leaf area per pot was also significantly affected by salt stress (Fig. 2), Bami cultivar having a higher leaf area than Hamedani. In either one of the cultivars, leaf area, did not significantly differ between control and 25 mM NaCl salt concentration. Sadeghi and Emam (2006) reported that a low level of salinity may not reduce grain weight in wheat even though leaf area and dry weight be reduced. Djilianov *et al* (2003) reported that leaf area was the most sensitive growth parameter in response to high salt levels (75 and 100 mM of NaCl) in alfalfa, leaf area being reduced by 92% when plants were subjected to 150 mM NaCl.

These results also showed that salt stress significantly affected plant height in both cultivars, however, the decrease was greater in Hamedani cultivar (Fig. 3). Hashemi and Hajrasoliha (2001) reported plant height decrease with increase in salinity level in 5 alfalfa cultivars.

There was a significant difference in RWC between the two cultivars from 75 to 125 mM NaCl salinity concentration levels (Fig. 4). Bami had the higher RWC than Hamedani at all salinity level treatments. Meloni *et al* (2008) showed that an increase in salinity from 0 to 100 mmol reduced RWC by 22% in *Schinopsis quebracho Colorado*.

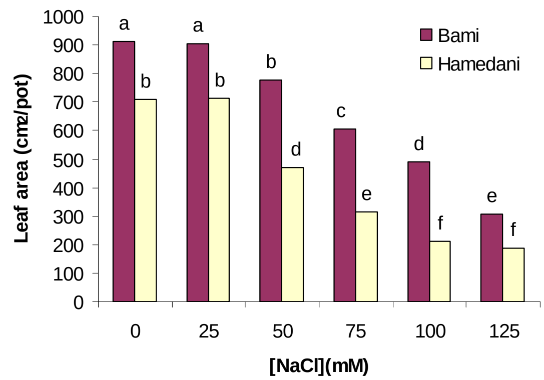

Fig. 2. Effect of NaCl concentration on leaf area per pot of two cultivars of alfalfa. Means followed by similar letters are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

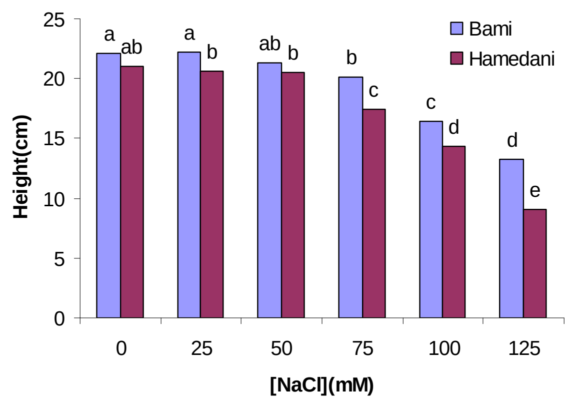

Fig. 3. Effect of NaCl concentration on height of two cultivars of alfalfa. Means followed by similar letters are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

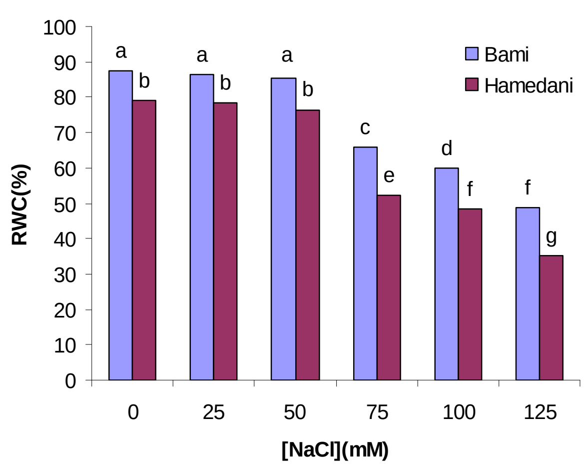

Fig. 4. Effect of NaCl concentration on leaf percentage Relative Water Content (RWC%) of two cultivars of alfalfa. Means followed by similar letters are not significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

3.2. Effect of salt stress on chemical composition of alfalfa

Results showed that K^+ accumulation decreased by increasing salinity level in either of the cultivars (Table 2). There were no significant differences observed among 0, 25 and 50 mM NaCl in either of the cultivars. In general, K^+ sequestration for Bami was higher than that for Hamedani at all NaCl concentration treatments (Table 2).

Salt stress also imposed a highly significant effect on Na^+ accumulation in both cultivars (Table 2). Na^+ accumulation increased by increasing the salinity level in either of the cultivars, howevere, Na^+ accumulation in Bami cultivar was lower than that in Hamedani probably due to Na^+ exclusion mechanisms in this cultivar. Maximum Na^+ accumulation (643 mmol kg^{-1}) was obtained at 125 mM salinity level for Hamedani cultivar.

By increasing salt stress, Ca^{2+} content was decreased, however, there were no significant differences observed among 0, 25, and 50 mM NaCl for Hamedani and 0 and 25 for Bami cultivars (Table 2). Suyama *et al* (2007) reported that Ca^{2+} content in alfalfa was decreased as the irrigation water salinity increased and Ca^{2+} contents at control level, 50 and 75 mM of NaCl were 328, 193, and 158 mmol kg^{-1}, respectively.

The results also indicated that increasing salinity from 0 to 125 mM of NaCl increased Cl^- content in both cultivars (Table 2). Bami and Hamedani cultivars responded similarly to salinity but Hamedani showed a higher amount of Cl^- content (Table 2).

K^+/Na^+ ratio decreased markedly at higher levels of salt stress, however, there was no significant difference observed between 0 and 25 mM NaCl in either one of the cultivars (Table 2). Bami cultivar contained a higher K^+/Na^+ ratio than Hamedani at all salt stress levels. Bami cultivar as compared to Hamedani when with the highest K^+/Na^+ ratio produced the greatest dry weight per pot (Table 2 & Fig. 1). It is very important for cells to maintain a low content of cytosolic Na^+ or to maintain a high K^+/Na^+ ratio in the cytosol when under salt stress (Maathuis and Amtmann 1999). Similar to K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio was decreased by increasing salt stress levels and there being highly significant differences from 25 to 125 mM NaCl in either one of the cultivars (Table 2). Plants maintained considerably higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots, with the former ratio being significantly higher than 1. A minimum level is suggested for normal functioning of plants under saline conditions (Wyn Jones 2001). Gholipor *et al* (2004) reported that under salt stress conditions, a Kabuli cultivar of chickpea carried a lower concentration of K^+ and Ca^{2+} as well as a lower K^+/Na^+ ratio with its dry matter weight decreased with increasing salinity. The maintenance of higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in shoots of ajowain (*Trachyspermum ammi* L.) could be an important indication of its salt tolerance, although both K^+/Na^+ and Ca^{2+}/Na^+ ratios consistently decreased in the shoots (of ajowain) with increase in salt level in the growth medium (Ashraf and Orooj 2006).

There was a significant positive correlation between dry matter weight per pot and K^+/Na^+ ratio for Bami (R^2=0.95) and Hamedani (R^2=0.94) cultivars under salt stress (Fig. 5). Also, Fig. 6 shows a strong positive relationship between dry weight and RWC% for both Bami (R^2=0.94) and Hamedani (R^2=0.96) cultivars under salt stress conditions.

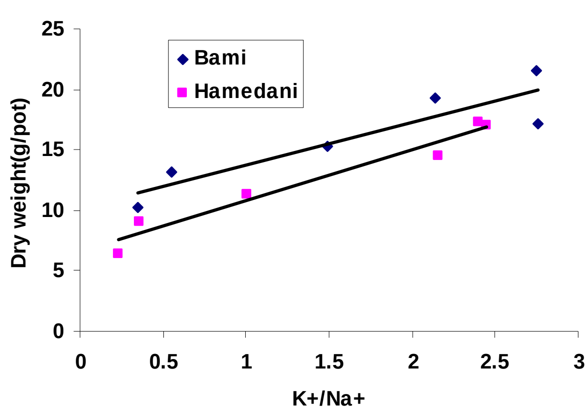

Fig. 5. Relationship between dry matter weight per pot and K^+/Na^+ ratio at salinity treatments for two cultivars of alfalfa

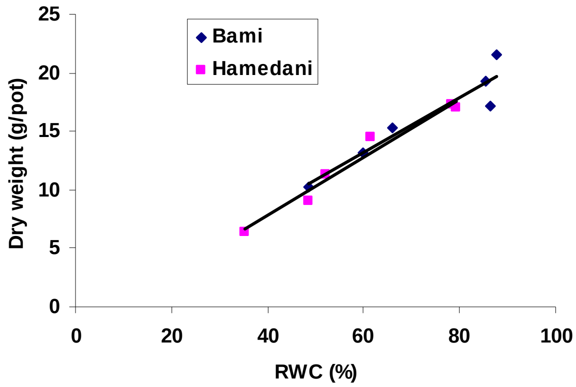

Fig. 6. Relationship between dry weight per pot and percentage leaf Relative Water Content (RWC%) at salinity treatments for two cultivars of alfalfa

4. Conclusion

It was found that in a glycophyte crop such as alfalfa, Na^+ and Cl^- content were increased, whereas K^+ and Ca^{2+} were decreased consistently with the progressive increase in salt level of the growth medium, however, the ability of the plants to tolerate a sudden increase in salt concentration depends on the rate of ion accumulation and the nature of the ion. Once NaCl has accumulated in the cells to a high level, the alfalfa plants are unable to recover. Alfalfa is a moderately sensitive-tolerant crop whose response to salinity is associated with maintenance of high K^+/Na^+ and Ca^{2+}/Na^+ ratios in shoots. All in all it seems that less adverse effects of salinity on Bami cultivar may make it more suitable for growth in saline soils than Hamedani cultivar. This subject is worthy of further study and explorations.

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