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Relationship Between Ion Accumulation and Plant Biomass of Alfalfa Under Salt Stress

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Abstract

Salinity is one of the major abiotic challenges influencing plant productivity worldwide. To examine the response of two alfalfa cultivars (Bami and Hamedani) to six levels of water salinity (0, 25, 50, 75, 100, 125 mM L^{-1} NaCl), a glasshouse experiment was conducted at the College of Agriculture, Shiraz University, Shiraz, Iran in 2008. The results showed that in Bami (which appeared to be more tolerant to salinity), with increasing salt stress from 75 to 125 mM L^{-1}, dry weight per pot was significantly decreased from 15.1 to 10.3 g, while in Hamedani decrease in dry weight was started from 50 mM L^{-1} salinity level. In both Bami and Hamedani cultivars leaf area per pot was significantly decreased when salt stress was higher than 75 mM L^{-1}. There was a significant difference in leaf relative water content (RWC) between the two alfalfa cultivars in 75, 100 and 125 mM L^{-1} NaCl salinity levels. With increase in salinity level, the Na^{+} concentration was increased from 220 to 565 mmol kg^{-1} dry weight in Bami, and from 238 to 643 mmol kg^{-1} dry weight in Hamedani. The Cl^{-} concentration in Hamedani (622 mmol kg^{-1} dry weight in 125 mM L^{-1} NaCl) was higher than that in Bami (503 mmol kg^{-1} dry weight in 125 mM L^{-1} NaCl). The K^{+} concentration was increased from 203 to 604 mmol kg^{-1} dry weight in Bami, and from 135 to 571 mmol kg^{-1} dry weight in Hamedani. Furthermore, phytomass production in Bami was significantly correlated with K^{+} (r=0.97), and Ca^{2+} (r=0.96) concentrations, as well as with leaf area (r=0.50) and plant height (r=0.87). Path analysis showed that there were significant direct effects of leaf area (p=0.73), Ca^{2+} (p=1.02) and K^{+} (p=0.59) on dry weight in Bami. In Hamedani, there were significant direct effects of K^{+} (p=0.61) and Ca^{2+} (p=1.07 on dry weight. Overall, based on both correlation and path analyses, it was concluded that measurements of K^{+} and Ca^{2+} concentrations may be appropriate criteria for evaluating susceptibility of cultivars to salt stress. These results may also be used for screening the salt resistant cultivars of alfalfa.

Keywords: Alfalfa, Correlation, Ion concentration, Path analysis, Salt stress

Introduction

One of the most important limiting factors which can cause major loss in crop productivity is soil and water salinization (Munns 2002; Ahmadi *et al.* 2009; Emam *et al.* 2009; Sadeghi and Emam 2006). Salt affected lands, which is around 7% of the world's land area, i.e. 930 million ha (Ashraf and Orooj 2006), are generally less productive and less profitable for salt-sensitive and often more valuable forage crops such as alfalfa (Djilianov *et al.* 2003). In Fars province of Iran, salt stress is considered to be a growing problem in irrigated agricultural areas with low water quality and poor soil drainage (Sadeghi and Emam 2006). In these areas, where the rainfall is often low, cropping of salt tolerant cultivars will allow crops to extract more water.

Cordivilla *et al.* (1999) reported that in legumes, increase in salt stress level, greater than 50 mM L^{-1} NaCl, was associated with decrease in growth and productivity. Hashemi and Hajrasuliha (2001) found that some alfalfa cultivars (Ranger and Rahnani) produced more dry weight and leaf area, compared with other cultivars (Maopa and Yazdi), under salinity levels greater than 50 mM L^{-1} NaCl. Safanejad *et al.* (1996) reported that there was a strong positive relationship between dry weight and RWC in alfalfa under salt stress conditions. Vaughan *et al.* (2002) declared that in MnPl-9-LF and MnPl-9-HF cultivars of alfalfa, with increasing salt stress from 70 to 300 mM L^{-1} NaCl a respective decline of 36 to 87% in dry weight production was occurred.

Salinity inhibits plant growth due to water deficit and or ion-excess effects (Munns *et al.* 2006), while higher amounts of sodium (Na^+) in plant tissue is toxic, but potassium (K^+) is essential to the plants. The cytosol of plant cells normally contains 100-200 mM L^{-1} of K^+ and 1-10 mM L^{-1} of Na^+. Under the typical salt stress conditions, accumulation of high Na^+/K^+ disrupts enzymatic functions that are normally activated in cells (Munns 2002; Munns *et al.* 2006). Under salt stress, it is important for cells to maintain a low concentration of Na^+ or to maintain a high K^+/Na^+ ratio in the cytosol (Maathuis and Amtmann 1999). Plant cells that maintained higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the shoots, can function normally under saline conditions (Jones 2001). Ashraf and Orooj (2006) reported that the maintenance of higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in shoots by ajowain (*Trachyspermum ammi* L.) could be an important component of its salt tolerance. Gholipor *et al.* (2004) showed that under salt stress conditions, the Kabuli cultivars of chickpea had lower K^+ and Ca^{2+} concentrations and K^+/Na^+ ratio and their dry weights decreased with increasing salinity level.

The major global problems with soil and salinity is caused by human activities, and Na^+ and Cl^- are the ions that are responsible for the plant yield losses (Ashraf and Orooj 2006; Ahmadi *et al.* 2009). Osmotic potential of the soil increases, when NaCl as well as other soil ions accumulate, and this obstructs the uptake of water by the plants. Further perturbations of plant metabolism occur when toxic ions such as Na^+ or Cl^- enter the plant cell (Suyama *et al.* 2007; Da Silva and Nogueira 2008).

The aim of this research was to examine the vegetative growth, ion accumulation and biomass production of two alfalfa cultivars under salinity stress and also to clarify the relationship between ion accumulation and biomass production of two alfalfa cultivars using correlation and path analyses.

Materials and Methods

In order to examine the effects of sodium chloride on vegetative growth and ions accumulation in two alfalfa cultivars (Bami and Hamedani), a greenhouse experiment was conducted during Spring and Summer 2008. Bami is a late season cultivar, with a plant height of up to 100 cm, high yield potential and vertical growth habit, which is adapted to temperate and semi-tropical environments of Fars Province. Hamedani is an intermediate season cultivar, with a plant height of up to 75 cm, medium yield potential and prostrate growth habit, which is adapted to cold and temperate environments of Fars Province (Bahrani 2000). The experiment was carried out at College of Agriculture, Shiraz University, Shiraz, Iran, on a fine mixed, mesic Typic Calcixerpets soil. There were six levels of salinity (i.e. control, 25, 50, 75, 100, and 125 mM L^{-1} NaCl). The temperature range was 25 to 30° C, with 70 to 80% relative humidity throughout the experiment. The light intensity was in the range of 600-1000 μmol m^{-2} s^{-1}. The experiment was arranged as completely randomized design with four replications. Physico-chemical properties of soil before sowing was as: sand, silt, and clay= 7, 67.6 and 25.4%, respectively, organic matter content= 0.91%, pH=7.1, EC=0.05 dS/m, total nitrogen=0.09%, phosphorous= 16.1 mg kg^{-1} and potassium= 463 mg kg^{-1}.

Alfalfa seeds were sown in 5 kg plastic pots filled with soil fertilized with 20, 25 and 10 mg kg^{-1} NPK, respectively. Before sowing, some gravel (5 to 10 mm) was put at the bottom of each pot to allow drainage. Fifty seeds were sown in each pot on April 28th 2008, and then thinned to twenty seedlings at the two-leaf stage and harvested at 10% flowering on July 3rd 2008. When the plants were at the two-leaf stage, salt treatments were applied. The pots were irrigated every five days up to field capacity and the EC of the soil in each pot was measured before and after each irrigation, using a portable EC meter (Model Scout 1010, UK).

Leaf area per pot was measured at harvest using a leaf area meter (Model 3000, LI-COR Inc.: Lincoln, NE) 67 days after NaCl treatment. At harvest, the forages were cut, rinsed briefly in de-ioinized water to remove surface salts and dust, weighted and samples were dried in an oven at 72° C for 48 hours, to determine dry weights. The dried tissues were ground to pass a 1 mm mesh in a Thomas-Wiley laboratory mill for Na^+, K^+ and Ca^{2+} quantification. The contents of Na^+, K^+ and Ca^{2+} were then determined using a flame photometer (Model Jenway PFP-7). Chloride was measured by titration after samples were oven-dried and ground material was extracted in de-mineralized water for 30 min (Chen *et al.* 2001). The leaf relative water content (RWC) was calculated according to Beadle *et al.* (1993) using the following equation:

$$RWC = [(FW - DW)/(TW-DW)] \times 100$$

where FW is the fresh weight, DW is the dry weight and TW is the turgid weight. The data were subjected to analysis of variance, and the means were compared using Duncan's multiple range test ($p \leq 0.05$). Path and correlation analyses were done using SAS (2000).

Results and Discussion

Analysis of variance**:** Based on Table 1, ion accumulation, dry weight, leaf area, plant height and RWC were significantly affected by alfalfa cultivars and salt stress. Interactions of cultivar × salt stress for Na^+, Ca^{2+} and Cl^- accumulations, dry weight, leaf area and RWC were also significant (Table 1).

Table 1. Analysis of variance for measured traits of two alfalfa cultivars under salinity stress conditions

Source of variations	Degrees of freedom	K+	Na +	Ca++	Cl-	Dry weight/ pot	Leaf area/pot	Height	%RWC
Cultivar	1	23999.2 *	6916.7 **	930.25**	37249.0*	148.03**	210834.0**	42.25**	1560.25**
Salt Stress	5	196891.5**	180770.1*	425.25*	151444.6**	113.69**	66313.9*	102.85**	1676.02**
Cultivar × Salt	5	1356. 8ns	2954.1**	45.25**	4823.8**	0.62*	7968.5 *	2.05ns	56.91**
Error	36	1748.9	5.5	13.58	0.0135	0.77	52931.5	0.91	1.38

^{ns},* and ** : not significant and significant at 0.05 and 0.01 probability levels, respectively.
RWC= Relative Water Content

Effect of salt stress on vegetative characteristics and RWC of alfalfa

In Bami, with increasing salt stress from 75 to 125 mM L^{-1}, dry weight per pot was significantly decreased from 15.1 g/pot to 10.3 g/pot, while in Hamedani this decrease was started from 50 mM L^{-1}, indicating that Bami might be more tolerant to salinity than Hamedani (Table 2). These results are in agreement with those of Hashemi and Hajrasuliha (2001) who found that in alfalfa, increasing salt stress levels greater than 200 mM L^{-1} killed the plants due to toxicity effects of NaCl. Suyama *et al.* (2007) also reported that due to poor salt tolerance of alfalfa, increase in salt stress levels (greater than 100 mM L^{-1}) was associated with plant dry weight reduction. Similar findings have been reported by Emam *et al.* (2009).

In both Bami and Hamedani cultivars leaf area was significantly decreased when salt stress levels exceeded 75 mM L^{-1} (Table 2). Again, Bami, which appeared to be more tolerant, had greater leaf area (Table 2). Djovic *et al.* (2003) found that at higher salt stress levels (more than 75 mM L^{-1}) leaf area of alfalfa was decreased dramatically. In both alfalfa cultivars, plant height was reduced by salt stress, however, such reduction was greater in Hamedani (Table 2). In a similar study, Vaughan *et al.* (2002) declared that the long term irrigation of alfalfa with saline water of more than 70 mM L^{-1} should not be recommended, due to sharp decrease in the root growth, plant height and phytomass production.

There was a significant difference in RWC between the two cultivars at 75 to 125 mM NaCl salinity levels (Table 2) and Bami (i.e. the tolerant cultivar) had greater RWC than Hamedani under all salinity levels. Workers have attributed the variation in RWC between the alfalfa cultivars, when plants were exposed to salt stress, into genetic variations (Jones 2001; Emam *et al.* 2009). Safanejad *et al.* (1996) reported that Salado alfalfa cultivar with higher RWC was more tolerant to salinity stress compared to other cultivars with lower RWC. Annad *et al.* (2000) declared that an increase in salinity level from 25 to 250 mM L^{-1} decreased the RWC from 38 to 3%. Also, a strong positive relationships between leaf area and RWC for both Bami (R^2=0.96) and Hamedani (R^2=0.95) cultivars were found under salinity conditions (Figure 1). In addition, Bami with higher RWC had greater dry weight and leaf area, compared to Hamedani under each salinity level (Table 2).

Table 2. Effect of NaCl on dry weight, leaf area, plant height and relative water content (RWC) of two alfalfa cultivars

Treatment		Dry weight (g/pot)	Leaf area (cm^2/pot)	Height (cm)	RWC (%)
Cultivar	Salinity (mM L^{-1})				
	0	21.6a	912a	22.1a	87.6a
	25	20.1ab	906a	22.2a	86.5a
Bami	50	19.3b	776b	21.3ab	85.3a
	75	15.1d	607c	20.1b	66.0c
	100	13.1e	489d	16.4c	60.0d
	125	10.3fg	305e	13.2d	48.7f
	0	17.2c	711b	21.0ab	79.2b
	25	17.3c	715b	20.6b	78.3b
Hamedani	50	14.5de	469d	20.5b	76.3b
	75	11.0f	313e	17.4c	52.1e
	100	9.1g	213f	14.3d	48.6f
	125	6.4h	189f	9.1e	35.2g

In each column means followed by the similar letters are not significantly different using Duncan's multiple range test ($p \leq 0.05$

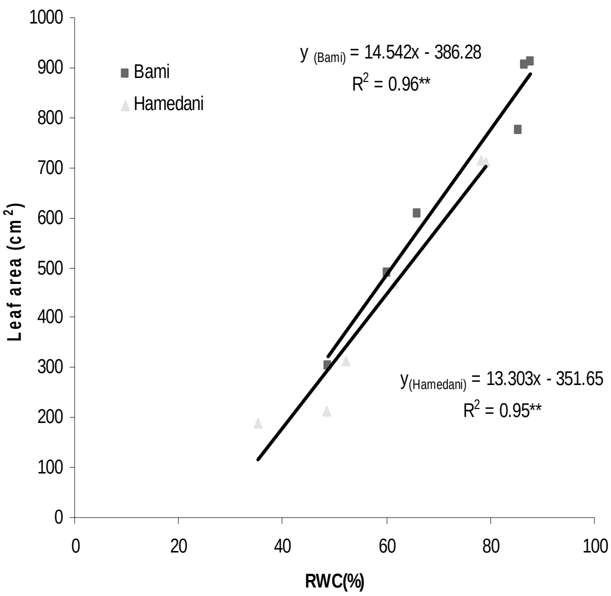


Figure 1. Relationship between leaf area and leaf relative water content (RWC) under salinity treatments for two alfalfa cultivars

Effect of salt stress on ion accumulation of alfalfa

Generally, in both cultivars, K^+ and Ca^{2+} concentrations of the foliage were decreased with increase in salt stress levels (Figures 2a and 2b). In Bami, K^+ and Ca^{2+} concentrations were higher than Hamedani under all stress levels. Jones (2001) suggested that the role of K^+ accumulation is more important than Ca^{2+} for cell growth and enzymatic activation under salt stress conditions. The Na^+ concentration was increased from 220 to 565 mmol kg^{-1} dry weight in Bami, and from 238 to 643 mmol kg^{-1} dry weight in Hamedani (Figure 2c). Also, with increase in salinity level, Cl^- concentration in Hamedani (622 mmol kg^{-1} dry weight at 125 mM L^{-1} NaCl) was higher than Bami (503 mmol kg^{-1} dry weight at 125 mM L^{-1} NaCl) (Figure 2d). Maathuis and Amtmann (1999) reported that it is very vital for plant cells to keep a low concentration of Na^+ and Cl^- and high concentration of K^+ and Ca^{2+} when exposed to salt stress. Similar results have been reported by other workers (e.g. Emam *et al.* 2009). Also, Bami was able to accumulate lower amount of Na^+ and Cl^- and higher amount of K^+ and Ca^{2+} than Hamedani (Figure 2).

Correlation

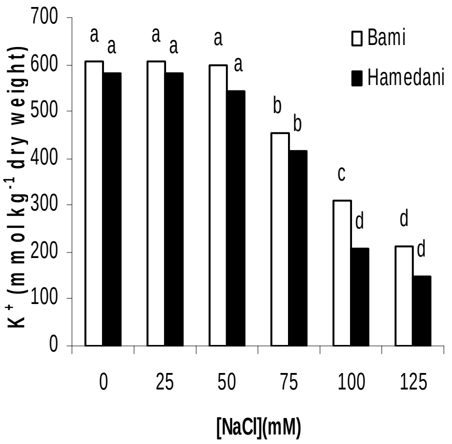
Results showed that in Bami, there were highly significant positive correlations between alfalfa dry weight and K^+ concentration (r=0.97), Ca^{2+} concentration (r=0.96), leaf area (r=0.50), alfalfa height (r=0.87) and RWC (r=0.98) (Table 3). On the other hand, there were significant negative correlations of alfalfa dry weight with Na^+ concentration (r=-0.91) and Cl^- concentration (r=-0.96) (Table 3). Results also revealed that in Hamedani, dry weight per pot was significantly correlated with K^+ (r=0.91), Ca^{2+} (r=0.95), leaf area (r=0.46) and RWC (r=0.98) (Table 4), while there was significant negative correlation between alfalfa dry weight and Na^+ (r=-0.95) and Cl^- (r=-0.98). These correlations confirmed the negative role of Cl^- and Na^+ in phytomass production of alfalfa.

In Bami, negative correlations between K^+ and Na^+ (r=-0.96), K^+ and Cl^- (r=-0.97), Ca^{2+} and Na^+ (r=-0.88) and Ca^{2+} and Cl^- (r=-0.99) were obtained (Table 3). Similar trends were observed for ion concentration in Hamedani (Table 4). It is worthy to note that according to Samonte *et al.* (1997), use of simple correlations may not be efficient for breeding purposes.

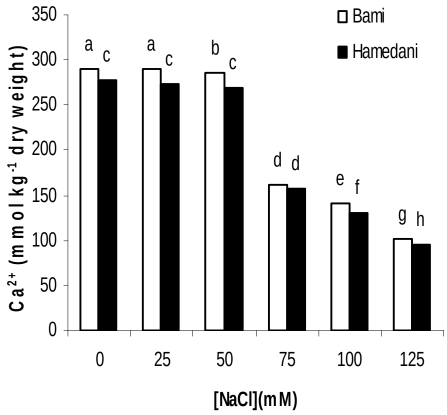
Path analysis

Based on path analysis in Bami, there were positive path coefficients, direct effects, between alfalfa shoot dry weight and leaf area (p=0.73), Ca^{2+} (p=1.02) and K^+ (p=0.59) (Figure 3a). In Hamedani, there were positive significant path coefficients between dry weight per pot and K^+ (p= 0.61) and Ca^{2+} (p=1.07), while a negative significant direct effect was found between alfalfa dry weight per pot and Na^+ (p=-1.01) and K^+ and Cl^- (p=-0.89) (Figure 3b). Many researchers have used path and correlation analyses in breeding programs of fodder oat (*Avena sativa* L.) (Bukhari *et al.* 2009), rice (*Oryza sativa* L.) (Rabiei *et al.* 2004), Cotton (*Gossypium hirsutum* L.) (Alishah *et al.* 2008) and wheat (*Triticum aestivum* L.) (Hui *et al.* 2008).

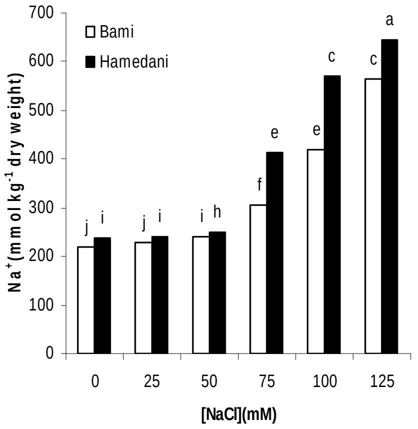
(a)



(b)



(c)



(d)

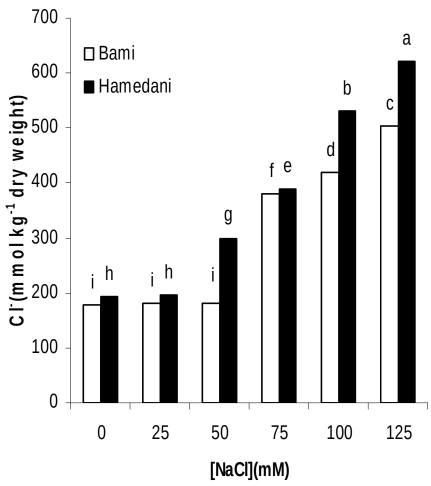


Figure 2. Effect of NaCl levels on K^+ (a), Ca^{2+} (b), Na^+ (c) and Cl^- concentrations (d) of two alfalfa cultivars

(Means followed by similar letters are not significantly different using Duncan's multiple range tests, $p \leq 0.05$).

Table 3. Correlation coefficients of measured traits for Bami cultivar

	K^+	Na^+	Ca^{++}	Cl^-	Leaf area/pot	Dry weight/pot	Height	RWC
K^+	1							
Na^+	-0.96**	1						
Ca^{2+}	0.96*	-0.88*	1					
Cl^-	-0.97**	0.89**	-0.99**	1				
Leaf area/pot	0.40*	-0.35	0.39*	-0.40	1			
Dry weight/pot	0.97**	-0.91**	0.96**	-0.96**	0.50*	1		
Height	0.94*	-0.95*	0.88*	-0.89*	0.23*	0.87**	1	
RWC	0.98*	-0.91*	0.99*	-0.99*	0.45**	0.98**	0.90*	1

* and ** significant at 0.05 and 0.01 probability level, respectively
RWC= Relative Water Content

In this study, there was a highly significant simple correlation of alfalfa dry weight with plant height in both cultivars (Tables 3 and 4), with Na^+ in Bami cultivar (Table 3) and with K^+ in Hamedani cultivar (Table 4). However, path coefficients were not significant for these traits in each cultivar (Figures 3 a and b), indicating that selection based on simple correlations may not be efficient as also reported by others (e.g., Samonte *et al*. 1997; Gravvois and Foyer 2002). The results of path and correlation analyses might be different since the two analyses evaluate different parameters (Campbell *et al*. 1980). Correlation measures mutual associations between variables, while path analysis shows relative importance of the association (Bukhari *et al.* 2009).

Generally, Bami had higher dry weight per pot, leaf area and plant height compared to Hamedani under salt stress conditions. Also, Bami with higher RWC had greater dry weight per pot than Hamedani. In addition, under salt stress levels greater than 75 mM L^{-1}, vegetative characteristics of both cultivars decreased significantly. Bami was able to maintain lower amount of Na^+ and Cl^- and higher amount of K^+ and Ca^{2+} than Hamedani. It appeared that Na^+ had toxic and negative effect on plant growth and ion balance, especially in Hamedani cultivar, under high salt stress. It was also concluded that evaluation of alfalfa cultivars based on both path and simple correlation coefficients might be more reliable than simple correlation alone.

(a) (b)

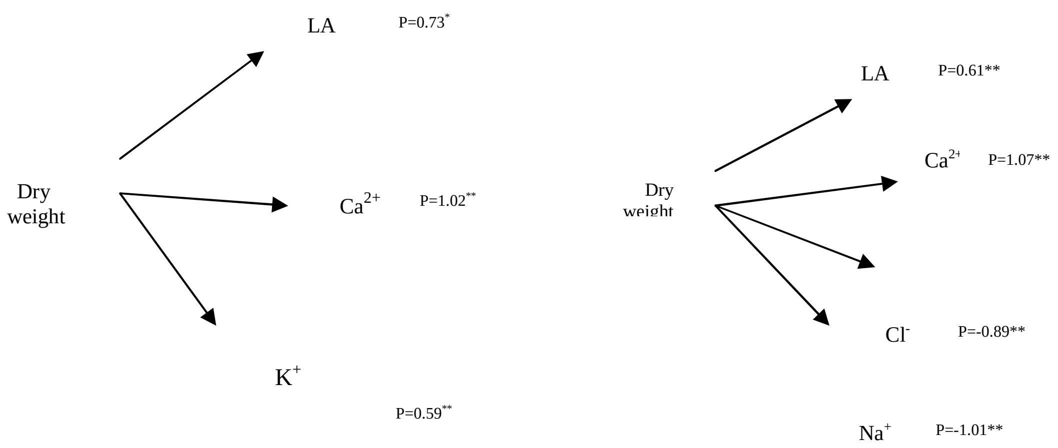


Figure 3. Diagram of path coefficients between dry weight and some measured traits in Bami (a) and Hamedani (b) cultivars

Table 4. Correlation coefficients of measured traits for Hamedani cultivar

	K^+	Na^+	Ca^{++}	Cl^-	Leaf area/pot	Dry weight/pot	Height	RWC
K^+	1							
Na^+	-0.96**	1						
Ca^{2+}	0.92*	-0.97**	1					
Cl^-	-0.95*	0.97*	-0.95**	1				
Leaf area/pot	0.41**	-0.38	0.38	-0.42	1			
Dry weight/pot	0.91**	-0.95**	0.95**	-0.98**	0.46**	1		
Height	0.92**	-0.95*	0.92*	-0.95*	0.30*	0.91**	1	
RWC	0.87*	-0.90**	0.91*	-0.96*	0.46**	0.98*	0.88*	1

* and ** significant at 0.05 and 0.01 probability level, respectively
RWC= Relative Water Content

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