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Changes of Seedling Growth and Ion Uptake of Chickpea Genotypes under Salt Stress Condition

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ABSTRACT

In this investigation, the effect of salinity on the germination and seedling growth stages of chickpea (*Cicer arietinum* L.) varieties in hydroponic condition was studied. The experiment was carried out as Factorial based on Completely Randomized Design (CRD) with 4 replications at the Physiology Lab at the Campus of Agriculture and Natural Resources, Razi University. The factors included different cultivars of chickpea (Jam, Bivanij, Arman, Azad, Hachem and ILC482) and different osmotic potential levels (0, 40, 80 and 120 mM) of NaCl. To study of micro and macro elements distribution in salinity condition, different parts of plant were evaluated. During these stages, the percentage of germination, total dry weight at seedling stage, root and shoot dry weight, chlorophyll fluorescence, stomatal resistance, and concentration of micro and macro elements were investigated. The results indicated that the interaction of variety and salinity for the most traits were significant. All of the traits except Na⁺ accumulation and stomatal resistance were decreased by increasing salinity concentration at germination and seedling growth stages. Arman and ILC482 at the germination stage and Hachem and Bivanij at seedling stage seem to have a greater tolerance to salinity stress.

KEYWORDS: chickpea, salinity, dry matter, stomatal resistance

INTRODUCTION

Chickpea is the most important pulse crop in the world after dry bean. Also, it is an important source of nutrition for humans and animals and helps to improve soil fertility, particularly in dry-lands farming. Chickpea similar to many other of leguminous crops is highly sensitive to salinity (Ashraf and Waheed, 1993). Salinity is one of the most important problems in the semi-arid and arid regions. Salinity leads to various metabolic disturbances resulting in general suppression of seed germination, plant growth, and yield of crops (Chandrasekar and Sandhayarani, 1996). It is estimated that about one third of the world's cultivated land is affected by salinity (Perez-Alfocea et al., 1996).

Generally, salt stress causes both osmotic stress and ionic stress. Salt stress that occurred in the soils with high salt reduces the ability of plants to absorb water, and this quickly reduces seedling growth (Munns, 2002). Ionic stress is caused by the over accumulation of salt in the cells (Ueda et al, 2003). Moreover, the effect of soil salinity on crops at seedling stage is higher than other growth stages, because seed germination usually occurs on the uppermost soil layers which accumulate soluble salts as a result of evaporation and capillary rise of water (Almansouri et al, 2001). Crop yield may be adversely affected by salinity as a result of nutritional disorders (Silva et al. (a) 2008). Salinity changes selective absorption of ions by roots and decreases translocation of these ions (Thomas, 1997). Salinity condition leads to nutritional imbalance on available elements, competitive absorption and translocation or distribution of elements. Although plants in nature have evolved several adaptive mechanisms to cope with the presence of salts in their environment (Zhou et al, 2009), the understanding of these mechanisms still remains incomplete. Thus, it can alter leaf water potential, stomatal conductance, and transpiration (Sultana et al., 1999; Parida and Das, 2005). The aim of this experiment was to evaluate the effect of salinity on germination characteristics and content of different mineral elements, chlorophyll flourcence and stomatal resistances in chickpea varieties.

MATERIALS AND METHODS

Experimental design and statistical analysis

The experiment was carried out as Factorial based on Completely Randomized Design (CRD) with 4 replications at the Physiology Lab at the Campus of Agriculture and Natural Resources, Razi University. Analysis was done using the MSTAT-C software. Differences between means were determined by Duncan's multiple range tests at 5% probability.

Six varieties of chickpea (*Cicer arietinum* L₂) namely Jam, Bivanij, Hachem, Azad, Arman and ILC 482 were used in the experiments. All the varieties were released from Agricultural Research Institute, Iran. Cultivar of Bivanij is the most commonly grown cultivar in Kermanshah region, Iran. The experiments were carried out

to assess total percentage of germination and seedling growth in response to salt levels. To meet the aim, two separate experiments were conducted:

Experiment I: Germination

Saline solutions were prepared artificially by dissolving calculated amount of NaCl with distilled water to make 40, 80, and 120 mM NaCl solutions, and distilled water served as the control. Chickpea seeds were carefully selected to avoid cracked seeds and were surface sterilized with $KMnO_4$ solution before experiment. Seeds were germinated in 9 cm Petri dishes with filter papers and 25 seeds per Petri dish were used for each treatment. 10 mL of the appropriate solution was applied on alternate days to each Petri dish covered and arranged randomly in incubators (23°C). The number of germinated seeds was counted after 7 days and data were recorded. Seed was considered as germinated seed when both plumule and radicle had emerged \geq 0.5 cm.

Experiment II: seedling growth stage

The experiment was conducted in a greenhouse at 22°C under 14h day length. Several plastic pots with 15cm diameter and 18cm deep were filled with 3 L tap water (each plastic tap). Five hundred seeds of each variety were germinated on moist filter paper in Petri dishes and 20 randomly chosen. Three days old seedling of each variety was transplanted and equidistant from each other into pot. The salinity treatments were 40, 80 and 120 mM NaCl with distilled water (control). The Pryanishnikov medium (Meychik and Yermakov , 2001) was dissolved. 7 ml of this medium was given to the tap water at the first day after planting and the other half (7 ml), was given 14 days after planting. The plants were harvested 20 days after the start of the experiment. Leaves, stems and roots were separated and dried at 70 °C for three days and then dry weights were recorded.

Dried samples of different parts of plant were powdered for chemical analysis. The concentrations of Fe^{2+} , Mn^{2+} , Zn^{2+} and Cu^{2+} were measured with Atomic Absorption Spectrophotometer (VARIAN-Cary 100 SCAN) after ashing at temperature about 500-550 °C and dissolving the ash into 20 % HCl. The concentrations of K^+ , Na^+ , N and P were measured after digestion with H_2SO_4 and H_2O_2 on a hotplate apparatus as follows: K^+ and Na^+ with flame photometer (PFP7, Jenway); P with spectrophotometer (UV-Visible, 100 scan, VARAN) and N measured by Kjeldahl method.

Leaf stomatal conductance was determined by Delta-T AP-4 porometer (2 days prior to harvest). Chlorophyll fluorescence emission from the upper surface of the leaves of intact plants was measured by a modulated fluorimeter (MiniPAM Photosynthesis Yield Analyzer, Walz, Effeltrich, Germany). Selected leaves for measurement of stomatal conductance were used for fluorescence measurements. The maximum quantum efficiency of PSII photochemistry (F_v/F_m) was assessed in leaves after 30 min of dark adaptation.

RESULTS AND DISCUSSION

Germination

The results indicated that interaction effect between varieties and salinity was significant for the traits of percentage of germination and shoot/root dry weight ratio (data not shown).

Percentage of germination: In this study, the cultivars of ILC482, Hachem and Arman showed the highest germination percentage in response to salinity at the highest level (Table 1). Increasing concentration of salt reduced significantly the percentage of germination. This was due to a reduction in absorption of water or toxic effects of certain ions on them, during the seed germination (Ashraf and Rasul, 1988). The detrimental effect of salinity on seed germination has received extensive attention (Esechie, 1995; Esechie et al., 2002; Soltani et al., 2002)

Shoot/Root dry weight: The difference between six cultivars in shoot/root dry weight ratio was significant and cultivars differed in their response to salinity. The salinity reduced S/R dry weight ratio at the high salt levels (Table 1). This result was different from the result that was reported by Ashraf and Rasul (1988). They reported that shoot/root ratio of mungbean cultivars increased at high salt levels, and Soltani et al., 2002 reported the shoot/root ratio of chickpea varieties was not significant.

Seedling growth stage

Dry weight determination

The results indicated that interaction effect between varieties and salinity was significant for the traits of stem, root and total (stem + root + leaf) dry weight (data not shown). The data for the stem biomass is given in Table 1. Arman produced significantly more stem dry weight than other varieties. Salinity, in general, damaged all plant parts of chickpea varieties. The root dry weight decreased progressively with the increasing salinity and the reduction was conspicuously greater in cultivar of Hachem than other varieties (Table 1).

Table 1. Interaction effects of salinity stress and cultivars of chickpea on characteristics of germination and seedling growth

Treatment	Percentage of germination	Shoot/Root dry weight	Stem dry weight	Root dry weight	Total dry weight			
0(mM)	(%)	ratio	(mg)	(mg)	(mg)			
ILC482	99.0ª	0.36 ^{bcd}	777.1 ^{def}	1494.0 ^{ab}	4242.0 ^{bc}			
Hachem	100.0 ^a	0.33 ^{cd}	664.7 ^{fghi}	1213.0 ^{cde}	3551.0 ^{de}			
Arman	99.0°	0.44 ^{abc}	1071.0 ^a	1556.0 ^a	5455.0°			
Azad	91.0 ^{ab}	0.35 ^{bcd}	768.7 ^{def}	1192.0 ^{de}	3772.0 ^{cde}			
Bivanij	72.0°	0.32^{cd}	952.7 ^{bc}	1542.0 ^a	4615.0 ^b			
Jam	94.0 ^{ab}	0.38 ^{abcd}	994.0 ^{ab}	1523.0 ^{ab}	4742.0 ^b			
40(mM)								
ILC482	100.0^{a}	0.34 ^{bcd}	619.0 ^{hij}	1116.0 ^{def}	3236.0 ^{efg}			
Hachem	100.0^{a}	0.34 ^{bcd}	365.3 ¹	540.0 ^{jk}	1716.0 ^h			
Arman	98.0 ^a	0.41 ^{abcd}	860.3 ^{cd}	1154.0 ^{def}	4263.0 ^{bc}			
Azad	85.0 ^{abc}	0.36 ^{bcd}	715.0 ^{efgh}	1231.0 ^{cde}	3604.0 ^{de}			
Bivanij	28.0^{d}	0.19 ^e	871.0 ^{cd}	1394.0 ^{abc}	4250.0 ^{bc}			
Jam	92.0 ^{ab}	0.49 ^a	821.7 ^{de}	1322.0 ^{bcd}	4119.0 ^{bcd}			
80(mM)								
ILC482	99.0 ^a	0.30^{d}	295.0 ¹	466.0 ^k	1564.0 ^h			
Hachem	98.0ª	0.33 ^{cd}	151. ^{3m}	208.0 ¹	734.7 ⁱ			
Arman	96.0 ^{ab}	0.37 ^{bcd}	630.0 ^{ghij}	858.3 ^{ghi}	3346.0 ^{ef}			
Azad	89.0 ^{abc}	0.35 ^{bcd}	558.0 ^{ijk}	1036.0 ^{efg}	3143.0 ^{efg}			
Bivanij	10.0 ^e	0.12 ^{ef}	735.3 ^{efg}	1403.0 ^{abc}	4257.0 ^{bc}			
Jam	79.0 ^{bc}	0.47 ^{ab}	670.7 ^{fgh}	1130.0 ^{def}	3523.0 ^{de}			
120(mM)								
ILC482	97.0ª	0.31 ^{cd}	107.7 ^{mn}	211.0 ¹	505.7 ^{ij}			
Hachem	92.0 ^{ab}	0.39 ^{abcd}	25.0°	68.0 ¹	139.3 ⁱ			
Arman	74.0^{abc}	0.37 ^{abcd}	524.3 ^{jk}	679.7 ^{ij}	2714.0 ^g			
Azad	72.0°	0.39 ^{abcd}	363.7 ¹	757.7 ⁱ	2001.0 ^h			
Bivanij	7.0 ^e	$0.06^{\rm f}$	490.3 ^k	973.3 ^{fgh}	2897.0 ^{fg}			
Jam	79.0 ^{bc}	0.40 ^{abcd}	467.3 ^k	826.3 ^{hi}	2654.0 ^g			
Means at least one common letter in each column, based on Duncan test at 5 percent level are not significantly different.								

Although Gill (1990) reported that root dry weight was less affected by salinity than stem and leaf in greengram. Similar results were reported by Rapran et al. (2001) and Gama et al. (2007). The total dry weight reduced by increasing salinity levels. The cultivars of Arman and Hachem showed the highest and lowest total dry weight at without salt stress condition and 40 mM salt stress. The reduction in total dry weight might be due to salinity which generally inhibits the growth of plants, through reduced water absorption. Reducing metabolic activities due to Na⁺ and Cl⁺ toxicity caused by ionic interference. Negative effects of salinity on plant growth had a direct effect on total dry weight accumulation. The decline in total dry weight under salinity was in agreement with previous findings (Gama et al.,2007; Silva et al., (b) 2008). In the present study, Bivanij and Hachem had the highest and lowest total dry weight in response to salinity in seedling growth stage at the highest salinity level, respectively (Table 1). Perhaps, differences in experimental methods, other environmental conditions and varieties were responsible for the differences in salinity tolerance.

Effect of salt stress on ion uptake

K⁺ accumulation

These results indicated that salinity stress had an adverse effect on K^+ uptake. The concentration of K^+ was significantly influenced by salt level and varieties (data not shown). The varieties showed a decrease in K^+ content in different plant parts. However, among the varieties, Bivanij, Azad and Hachem showed the highest concentration of K^+ in leave, stem and root, respectively (Table 2). As a result of salinity, therefore, potassium accumulated in shoot rather than root by salinity effect.

Table 2. The effect of salinity on Ion accumulation at different parts of chickpea cultivars

			•				
K ⁺ (%)	Leaf	Stem	Root	Ca ²⁺ (%)	Leaf	Stem	Root
ILC482	9.9 ^c	$8.4^{\rm d}$	6.8^{f}	ILC482	3.5 ^{ef}	1.9 ^j	2.9 ^{gh}
Hachem	10.1°	6.9 ^f	7.8 ^e	Hachem	3.7 ^{cde}	2.4 ⁱ	3.5 ^{ef}
Arman	6.5 ^f	5.5 ^g	4.4 ^h	Arman	4.1 ^{bc}	3.9 ^{cd}	4.5 ^{ab}
Azad	10.2 °	9.9°	6.6 ^f	Azad	4.7 ^a	3.1 ^{ef}	3.6 ^{de}
Bivanij	13.2 a	3.1^{i}	5.7 ^g	Bivanij	3.3 ^{ef}	2.7^{hi}	3.1 ^{fg}
Jam	11.6 ^b	3.1 ⁱ	2.3 ^j	Jam	1.5 ^j	2.5 ^{hi}	$4.0^{\rm cd}$
Na ⁺ (%)				P (%)			
ILC482	9.5 ^f	11.4 ^d	12.1°	ILC482	4.4 ^d	3.2 ^{gh}	5.1 ^a
Hachem	14.4 ^b	14.1 ^b	16.5 ^a	Hachem	$3.4^{\rm g}$	2.9^{i}	$3.8^{\rm f}$
Arman	6.6 ⁱ	7.2 ^{hi}	9.4 ^f	Arman	4.1 ^e	2.9^{i}	4.5°
Azad	8.6 ^g	9.9 ^{ef}	16.3 ^a	Azad	$3.8^{\rm f}$	2.8i	4.6°

Bivanij	10.5 ^e	9.8 ^f	11.5 ^{cd}	Bivanij	4.3 ^{de}	2.9 ⁱ	4.6°
Jam	8.7 ^g	7.7 ^h	9.4 ^f	Jam	3.7 ^f	3.2 ^h	4.8 ^b
N (%)				Fe ²⁺ (ppm)			
ILC482	12.1 ^a	6.3 ^{fg}	11.6 ^b	ILC482	249.5 ^{ef}	255.2 ^{ef}	601.9 ^{cd}
Hachem	9.2°	6.9 ^e	6.8 ^e	Hachem	209.4 ^f	239.5 ^{ef}	558.2 ^d
Arman	6.0^{gh}	3.3 ⁿ	4.3 ¹	Arman	199.9 ^f	249.5 ^{ef}	610.4°
Azad	5.8 ^{hi}	3.4 ⁿ	4.6 ^l	Azad	215.2 ^{ef}	250.3 ^{ef}	268.3 ^e
Bivanij	5.1 ^k	3.9^{m}	6.4^{f}	Bivanij	211.2 ^{ef}	242.3 ^{ef}	941.9 ^b
Jam	7.9^{d}	5.3 ^{jk}	5.6 ^{ij}	Jam	207.4 ^f	252.8 ^{ef}	1016.0 ^a
Zn ²⁺ (ppm)	Cu ²⁺ (ppm)						
ILC482	39.5 ^{ij}	54.2 ^j	35.0^{f1}	ILC482	12.4 ^e	16.6 ^d	20.0 ^b
Hachem	22.9 ^j	31.9 ^j	31.9 ^j	Hachem	1.9 ⁱ	5.6 ^{gh}	18.6 ^b c
Arman	69.9 ^h	140.3 ^f	295.7 ^a	Arman	13.2 ^e	10.4 ^f	26.4 ^a
Azad	116.0 ^g	168.1 ^e	272.3 ^b	Azad	3.9 ^h	12.1 ^{ef}	25.9 ^a
Bivanij	115.1 ^g	150.2 ^f	230.7°	Bivanij	7.4 ^g	13.3 ^e	17.0 ^{cd}
Jam	139.4 ^f	150.3 ^f	192.5 ^d	Jam	4.8 ^h	7.4 ^g	19.6 ^b
Means at least one common letter in each column, based on Duncan test at 5 percent level are not significantly different.							

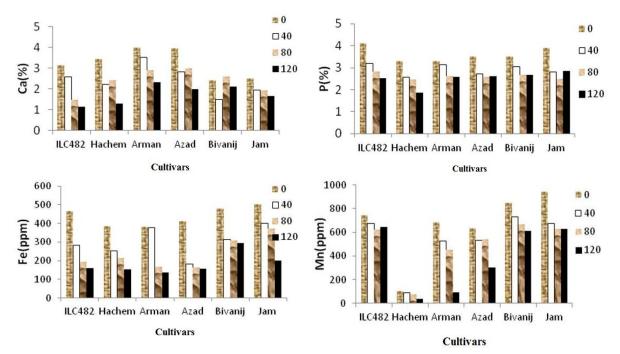


Figure 1. The effect of salinity on concentration of Ca²⁺, P, Fe²⁺ and Mn²⁺ ions in different cultivars of chickpea.

Under saline conditions plant cells utilize K^+ as a metabolic to maintain pressure turgor to escape from osmotic shock, so this K^+ ion selectively accumulated in the leaf chloroplast in response to increase salinity in order to maintain photosynthetic activity. On an average, Bivanij and ILC482 accumulated greater amount of K^+ than other varieties at all levels of salinity.

Ca²⁺ and Na⁺ accumulation

The concentration of Na⁺ increased significantly with increasing salinity level in all chickpea varieties. The difference among the varieties and salinity levels and their interaction was significant for Na⁺ concentration (data not shown). Also, Na⁺ accumulation in roots was greater than leaves (Table 2). The cultivars of Hachem and Azad showed the most accumulation of Na⁺ concentration as compared to other varieties in root (Table 2). Most tolerant legumes exclude Na⁺ or Cl⁻ ions from leaves to roots in salinity condition. These results are in agreement with results of Mohamedin et al. (2006) and Khorshidi et al. (2009).

Increasing Na⁺ absorption inhibited Ca²⁺ concentration in all varieties (Fig 1). Decreasing of Ca²⁺ accumulation in salinity treatment of 120 mM was 83 percent of the control in 'ILC482' cultivar but only 12.39 percent in 'Bivanij'. Apparently, roots of chickpea varieties were not capable to increase translocation of Ca²⁺ ion to the leaves. Khorshidi et al. (2009) reported that tolerant alfalfa cultivars can absorb more K⁺ and Ca²⁺ ions under saline condition and prevent Na⁺ absorption with a subsequent increase in K⁺/Na⁺ and Ca²⁺/Na⁺ ratios.

P accumulation

The NaCl treatments decreased P concentrations in the shoot and root. P accumulation in 'Jam' was significantly higher than other cultivars at the highest salinity level (Fig1). Decline in the P uptake can be ascribed to restricted mobility in the root growth zone caused by salinity stress. As reported by Mohamedin (2006), decreasing P availability was not only controlled by ionic bonds, which decreased P activity, but Ca-P complexes controlled P dissolution; but in some experiments P uptake was increased or not affected in saline condition (Yahya,1998; Turan et al., 2010).

N and Fe^{2+} accumulation

The concentration of N decreased in all cultivars by increasing salinity levels. The different response of N in shoots and roots on N uptake and reduction are related with a specific effect of Cl on N transport that it could be related with the reduction of malate by Cl⁻ ion because malate is a highly active ion in the N transport. N concentration in leaves was significantly greater than those found for the roots. The cultivar of ILC482 showed the highest N accumulation in leaves (Table 2). The similar result was reported by (Ahmad et al., 2005).

Our results also showed that Fe^{2+} uptake was strongly affected by increasing salinity levels. Fe^{2+} concentration significantly decreased with increasing salinity levels in all cultivars. In control condition, Jam had higher accumulation than other cultivars (Fig 1). The roots showed the most accumulation in compared to other plant parts. The cultivars of Jam and Azad had the highest and lowest Fe^{2+} accumulation in their roots, respectively (Table 2).

Zn²⁺ and Mn²⁺ accumulation

In the present study, applied NaCl due to decreasing Zn^{2+} uptake in chickpea cultivars and more accumulation of this ion happened in roots. The cultivar of Arman showed the highest Zn^{2+} in root than other cultivars (Table 2). Decreasing uptake of Zn^{2+} might also have resulted to higher pH in the substrate which ultimately resulted in poor uptake of this nutrient (Ghosh et al., 1987). Similar results were reported by (Mohamedin et al., 2006 and Dravid and Goesami, 1987).

Salinity has a negative interference on the manganese absorption. Applying NaCl decreased manganese concentrations in chickpea varieties (Fig 1). The amount of manganese absorption differed between plant genotypes. Our findings confirm the findings of Dhanda et al. (2004) in wheat, while in other researches manganese decreased or remained unchanged (Tuna, 2008).

Stomatal resistance

In the present study, the measured stomatal resistance at the end of the experimental period showed that under salinity, chickpea plants closed their stomatal which leads to enhanced stomatal resistance. The cultivar of ILC482 showed the highest stomatal resistance (Fig. 2). Similar results were reported by Turan *et al.* (2010). Stomatal closure is known to be an effective mechanism for economical water utilization under salt stress and limitation of the harmful salt ions uptake (Hasegawa *et al.*, 2000).

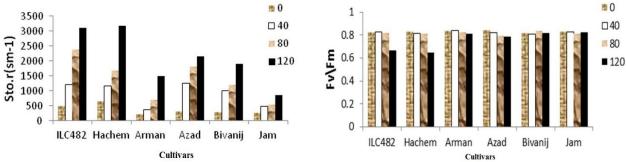


Figure 2. The effect of salinity on stomatal resistance and Fv/Fm traits of chickpea cultivars.

Chlorophyll fluorescence

In this study, No significant changes in the maximum quantum efficiency of PSII photochemistry (F_{ν}/F_{m}) occurred under 0, 40, 80 and 120 mM NaCl. Salinity seems not to affect PSII primary photochemistry. The most chickpea cultivars didn't show the significant variations in salinity levels (Fig 2). Similar results have been reported by Brugnoli and Björkman (1992).

CONCLUSIONS

The use of laboratory (Petri dish) germination as an estimation of seed viability is a standard practice. However, laboratory germination using saline irrigation may not give an accurate estimate of seedling emergence in the field (Eschie, 2002). The results of the experiment showed that ion uptake was strongly affected by salinity treatment. The association between osmotic and ionic effects (ionic toxicity, nutritional deficiency and/or imbalance) has been reported as being the main reason of the growth reduction under salt stress (Mohamedin et al. 2006). Measurement of stomatal resistance provides a sensitive tool for determining the degree of stress in plants. Reducing in leaf water potential will reduce stomatal conductance and eventually inhibit photosynthetic metabolism (Baker and Rosenqvist, 2004). Measurement of chlorophyll fluorescence has been developed as one of the most frequently used measuring tools in the basic photosynthesis research. The results showed that there wasn't significantly different between the cultivars and salinity stress for the maximum quantum efficiency of PSII (Fv/Fm) trait.

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