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Variability in the Response of Chickpea Cultivars to Short-Term Salinity, in Terms of Water Retention Capacity, Membrane Permeability, and Osmo-Protection

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Abstract: Seedlings of 2 chickpea cultivars (*Cicer arietinum* L.), salt-tolerant kabuli (CSG 9651) and salt-sensitive desi (DCP 92-3), were raised under control (distilled water) and salinity (EC = 4, 6, and 8 dS m^{-1}) conditions. Salt treatments were applied once symbiosis was well established (i.e. 15 days after sowing [DAS]) and continued until the last sampling stage (i.e. 70 DAS). The experiments were terminated 70 DAS and the plants were analyzed 40 and 70 DAS. Salt stress decreased the relative water content (RWC), membrane stability index (MSI), leaf chlorophyll (CHL), plant biomass, and grain yield, and increased total soluble sugars in both cultivars at both stages (40 and 70 DAS). Salinity-induced declines in RWC, MSI, CHL, biomass, and grain yield were significantly greater in desi DCP 92-3 than in Kabuli CSG 9651. DCP 92-3 also had higher accumulation of Na^+ in the roots as well its translocation into shoots, which had a negative impact on the K^+/Na^+ ratio. Results indicate that the salinity tolerance of kabuli CSG 9651, as manifested by less of a decrease in biomass and grain yield, was associated with higher membrane permeability, osmolyte concentration, and potassium content, and lower sodium content, as compared to salt-sensitive desi DCP 92-3. It is apparent that the salt-tolerant cultivar had better protection against salt-induced stress as a result of the cumulative action of various physiological and biochemical processes.

Key Words: *Cicer arietinum*, cultivars, membrane stability index, osmolytes, relative water content

Introduction

Soil salinization is a major constraint limiting agricultural productivity, particularly in arid and semi-arid regions. Salinity affects at least 20% of the world's arable land and more than 40% of irrigated land, to various degrees (Rhoades and Loveday, 1990). Salt-induced inhibition of plant growth may be due to an elevation in osmotic pressure and toxicity of specific ions, which may disturb the plant-water relationship or influence cellular physiological and metabolic pathways (Hasegawa et al., 2000). An important consequence of salinity stress in plants is ion transport, compartmentation, synthesis, and accumulation of osmotic solutes (Viegas et al., 2001). Salinity also results in growth retardation and reduction in fruit size, and decreases the number and size of seeds, and consequently yield (Ansari et al., 1998). As a consequence of these primary effects, secondary stresses, such as oxidative damage, often occur (Sairam et al., 2002). Changes in relative water content, osmotic adjustment, and ion exclusion are considered important mechanisms for salt tolerance in plants.

Chickpea (*Cicer arietinum* L.), one of the most important pulse crops, is a relatively salt-sensitive legume (Lauter and Munns, 1986). Because of the inherent sensitivity of chickpea plants to salinity, salt stress is a major constraint to their growth and yield. Chickpea is classified into desi and kabuli types, based primarily on seed color. Because the genus is indigenous to arid areas,

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some cultivars may have a degree of salt adaptation (Soussi et al., 1999; Rao et al., 2002); however, whether or not the differences in salt tolerance between cultivars are mainly due to genetic or morphologic factors has yet to be determined. Developing varieties with greater salt tolerance can improve productivity. A better understanding of the dominant effects involved in plant responses to salinity will facilitate development both of improved varieties and crop management practices.

Therefore, the objective of the present study was to evaluate the effects of salinity in chickpea cultivars differing in their sensitivity to salt stress, in terms of physiological and biochemical mechanisms of salt tolerance, and to correlate these mechanisms with changes in ionic and organic solute accumulation in order to better understand the mechanism of salt tolerance in these cultivars.

Materials and Methods

Seeds of *Cicer arietinum* L. cultivars DCP 92-3 (desi) and CSG 9651 (kabuli) were selected after preliminary investigation of 10 cultivars obtained from the Central Soil Salinity Research Institute (CSSRI), Karnal, India. Seeds were inoculated with 1 ml (about 10^9 cells) of a log phase culture of salt-tolerant *Mesorhizobium ciceri* strain F: 75 procured from IARI, New Delhi. The seeds were surface sterilized with 30% (w v^{-1}) mercuric chloride for 2 min, then washed with sterile water and germinated in earthenware pots lined with polythene bags. Each pot was filled with garden loam and soil mixed with farmyard manure (7 kg in each pot) in the ratio of 2:2:1. Plants were subjected to saline stress by adding a salt solution (NaCl, Na_2SO_4, and $CaCl_2$ in the ratio of 7:2:1), and the electrical conductivity (EC) of different salinity levels [4 (S_1), 6 (S_2), and 8 (S_3) dS m^{-1}] was adjusted using an EC meter. The controls (S_0) were irrigated every other day only with tap water to maintain the optimum moisture level. Plants subjected to saline stress were irrigated with a saline solution fortnightly to maintain the desired level of salinity. Three plants of uniform size were maintained in each pot in the greenhouse. Plants were exposed to a normal day length (12-h photoperiod), natural temperature (22-18 °C, day and night), and 55%-70% relative humidity throughout the experimental period. Two pots, each with 3 plants, were sampled per treatment and were analyzed 40 and 70 days after sowing (DAS).

Leaf relative water content (RWC) was estimated according to the method of Whetherley (1950). Leaf material was weighed (0.5 g) to determine fresh weight, placed in double-distilled water for 4 h, and then turgid weight was recorded. Finally, the samples were dried in an oven at 65 °C for 48 h to determine dry weights. RWC was calculated as:

RWC = [(fresh weight – dry weight)/(turgid weight – dry weight)] × 100.

The membrane stability index (MSI) was determined according to Sairam et al. (2002). Leaf samples (0.1 g each) were placed in 10 ml of double-distilled water in 2 sets. One set was kept at 40 °C for 30 min and its conductivity recorded (C1) using a conductivity meter. The second set was kept in a boiling water bath (100 °C) for 15 min and its conductivity was also recorded (C2). The membrane stability index (MSI) was calculated as:

MSI = [1– (C1/C2)] × 100.

Leaf material (0.1 g each) of young, fully expanded leaves of 3 plants from each replicate were used for pigment extraction. Extraction of leaf chlorophyll was performed in dimethyl sulfoxide (DMSO), following the method of Hiscox and Israelstam (1979). The absorbance value of chlorophyll in DMSO was measured at dual wavelengths of 645 and 663 nm with a spectrophotometer, using DMSO as the blank.

Total free amino acids were extracted from leaf tissues (100 mg) and determined according to the method of Lee and Takahashi (1966), using ninhydrin reagent. The purplish blue color was read at 570 nm and the quantity of amino acids was calculated from a reference curve prepared using glycine.

Total soluble sugars were quantified from dried leaf tissues (100 mg) using the anthrone-sulfuric method of Yemm and Wills (1954). For calculation of sugars concentration, a standard curve was prepared in glucose.

Potassium and sodium content in the roots and shoots were estimated using flame photometry, according to Chapman and Pratt (1961). Two 5-g ground samples of roots and shoots were treated with a digestion mixture consisting of nitric acid, sulfuric acid, and perchloric acid in the ratio of 9:4:1.

All the presented values are the means of 6 replicates per treatment; the data were subjected to analysis of variance and the means were compared by the LSD test.

Results

In the present study (Table 1) RWC decreased in both cultivars as salinity increased; however, CSG 9651 was able to maintain a higher RWC at both stages under salinity conditions, as compared to DCP 92-3. CSG 9651 maintained 71% RWC at the highest salinity dose at 70 DAS, as compared to 53.2% for DCP 92-3. MSI was higher in the salt-tolerant genotype CSG 9651 than in DCP 92-3. MSI was 85.6 and 63.0, respectively, at the highest salinity dose of 8 dS m^{-1} at 70 DAS for CSG 9651 and DCP 92-3. Chlorophyll content declined significantly in both chickpea cultivars as a result of increasing salinity; the decrease being greater in DCP 92-3. CSG 9651 was able to withstand salt stress to a considerable extent and chlorophyll pigments did not show any significant negative effects at the salinity levels of 4 and 6 dS m^{-1}; however, the highest salinity dose of 8 dS m^{-1} caused a drop of 12.6% at 70 DAS. In DCP 92-3 saline concentrations of 6 and 8 dS m^{-1} were more injurious and reduced the chlorophyll content by 34.7% and 40.2%, respectively, at 70 DAS.

Free amino acids content accumulated in cultivar CSG 9651 under low saline conditions (4 and 6 dS m^{-1}), although the highest salinity condition proved detrimental (Table 2). In DCP 92-3, only the 4 dS m^{-1} condition brought about an accumulation of free amino acids, whereas 6 and 8 dS m^{-1} proved detrimental to amino acid synthesis. Total soluble sugars increased in both cultivars with increases in salinity level and time. CSG 9651 had a higher level of total soluble sugars in response to the control treatment and at the 3 salinity levels at all stages, as compared to DCP 92-3. DCP 92-3 had a negligible accumulation of soluble sugars in response to treatments of 4 and 6 dS m^{-1}, and the data were similar to those of the control plants.

Potassium content decreased with increased salt stress in both chickpea cultivars, which significantly disturbed the Na^+/K^+ ratio. The decline in potassium content was by 5.5% (CSG 9651) and 12.7% (DCP 92-3) in response to the 4 dS m^{-1} salt treatment at 40 DAS, which increased to 7.2% (CSG 9651) and 18.7% (DCP 92-3) at 70 DAS. The sodium content increased in both cultivars, but the

Table 1. Effects of salinity on RWC, CHL content, and MSI in chickpea cultivars.

Parameters	Salt treatment	CSG 9651		DCP 92-3	
		40 DAS	70 DAS	40 DAS	70 DAS
RWC (%)	S_0	80.4	78.0	71.0	69.1
	S_1	78.2	76.5	68.9	67.0
	S_2	75.6	75.0	63.7	64.5
	S_3	72.3	71.0	57.8	53.2
MSI	S_0	90.6	100	83.75	90.0
	S_1	88.9	98.34	73.05	80.0
	S_2	85.4	90.78	62.37	78.0
	S_3	81.4	85.63	50.41	63.0
CHL	S_0	11.415	12.017	14.502	15.488
Content (mg g^{-1} fw)	S_1	10.878	11.066	12.322	11.363
	S_2	10.778	10.704	10.240	10.100
	S_3	10.319	10.500	9.140	9.255

S_0 = control, S_1 = 4 dS m^{-1}, S_2 = 6 dS m^{-1}, S_3 = 8 dS m^{-1}.
LSD (5%) for RWC due to age: 0.40; treatment: 0.62; interaction: 0.78.
LSD (5%) for MSI due to age: 0.34; treatment: 0.50; interaction: 0.61.
LSD (5%) for CHL due to age: 0.29; treatment: 0.44; interaction: 0.59.

Table 2. Effect of salinity on total soluble sugars (TSS) and total free amino acids content in chickpea cultivars.

Parameters	Salt treatment	CSG 9651		DCP 92-3	
		40 DAS	70 DAS	40 DAS	70 DAS
TSS	S_0	30.0	40.5	11.1	16.3
(µg mg^{-1} dwt.)	S_1	33.6	47.8	12.0	18.2
	S_2	36.2	57.6	12.6	18.8
	S_3	39.0	58.2	13.2	19.9
Amino acid (Aa)	S_0	3.50	6.00	1.92	2.50
Content (µg mg^{-1} dwt.)	S_1	4.25	7.85	2.37	3.70
	S_2	4.77	9.50	1.62	2.00
	S_3	2.95	6.55	1.35	1.62

S_0 = control, S_1 = 4 dS m^{-1}, S_2 = 6 dS m^{-1}, S_3 = 8 dS m^{-1}.
LSD (5%) for TSS due to age: 0.44; treatment: 0.54; interaction: 0.83.
LSD (5%) for Aa due to age: 0.45; treatment: 0.56; interaction: 0.85.

values were significantly higher in DCP 92-3 than in CSG 9651. With the salinity level of 4 dS m^{-1}, the sodium content increased by 34.5% and 54.0% in CSG 9651, and 81.6% and 94.9% in DCP 92-3 at 40 and 70 DAS, respectively. As can be seen, the values in Table 3 show that the sodium/potassium ratio increased as salinity increased, at both stages of development in both cultivars.

The present study shows that salt stress led to a significant reduction in yield parameters and yield in both cultivars (Table 4). The percent of reduction in pod and seed numbers was more dramatic, which resulted in a reduction in seed weight per plant, and, ultimately, plant biomass. The highest concentration salt treatment (8 dS m^{-1}) had a negligible effect on plant biomass in the tolerant cultivar CSG 9651 (12.9% biomass loss), but was detrimental to the susceptible cultivar DCP 92-3, which suffered significant loss in plant biomass (52.0%). The harvest index was calculated and was also comparable to the data on the cultivars' yield characters, decreasing progressively with increasing levels of salt. DCP 92-3 had a harvest index at the highest salinity level of only 0.35 in comparison to 0.57 for CSG 9651 at 70 DAS.

Discussion

The present study shows that RWC decreased with increasing salinity stress in both cultivars. In different legumes, such as alfalfa (Serraj and Drevon, 1998; Nandwal et al., 2000) and mungbean (Kabir et al., 2004), decreased RWC under salt stress conditions was reported. Reductions in RWC under salinity stress may be attributed to decreased water uptake due to low substrate water potential, or to injury to the root system.

Results of the present study show that CSG 9651 maintained greater membrane stability than DCP 92-3. These results are in agreement with those reported by Sairam (1994), Sairam and Srivastava (2001), and Sairam et al. (2002), in which tolerant genotypes of wheat had higher MSI values than susceptible genotypes. Salinity stress caused an increase in membrane permeability and, according to Sairam and Saxena (2002), plant species respond differently to oxidative injury as a result of variations in their antioxidant systems under stress conditions.

In the present study salinity stress resulted in decreased chlorophyll content. Saline stress led to leaf chlorosis, which ultimately resulted in significant photo-inhibition and photodestruction of chlorophyll pigments. Similar results have been reported for other legumes (Ashraf, 1989; Sudhakar et al., 1991; Soussi et al., 1998; Al-Khanjari et al., 2002). The observed inhibitory effects of salt on chlorophyll might have been due to suppression of the specific enzymes responsible for the synthesis of green pigments (Strogonove et al., 1970),

Table 3. Effect of salinity on sodium and potassium content, and the sodium/potassium ratio in the shoots of chickpea cultivars.

Parameters	Salt treatment	CSG 9651		DCP 92-3	
		40 DAS	70 DAS	40 DAS	70 DAS
Sodium Content (mg g^{-1} dwt.)	S_0	1.62	2.85	2.18	3.36
	S_1	2.16	4.39	3.96	6.55
	S_2	2.71	5.40	5.00	8.39
	S_3	3.12	6.88	5.51	10.0
Potassium Content (mg g^{-1} dwt.)	S_0	4.65	5.23	3.44	4.55
	S_1	4.39	4.85	3.00	3.78
	S_2	4.28	4.66	2.76	3.51
	S_3	4.15	4.41	2.56	3.20
Na^+/K^+ Ratio	S_0	0.34	0.54	0.63	0.73
	S_1	0.49	0.90	1.32	1.73
	S_2	0.63	1.15	1.81	2.39
	S_3	0.75	1.56	2.15	3.12

S_0 = control, S_1 = 4 dS m^{-1}, S_2 = 6 dS m^{-1}, S_3 = 8 dS m^{-1}.
LSD (5%) for sodium content due to age: 0.22; treatment: 0.34; interaction: 0.49.
LSD (5%) for potassium content due to age: 0.13; treatment: 0.29; interaction: 0.47.

Table 4. Effect of salinity on plant biomass and yield components in chickpea cultivars.

Salt Treatment	Pods number per plant	Seeds number per plant	Seed wt. per plant (g)	Plant Biomass (g)	Harvest Index
			CSG 9651		
S_0	16.00	24.00	5.00	7.58	0.65
S_1	15.60	22.00	4.40	7.30	0.60
S_2	15.20	21.00	4.10	6.84	0.59
S_3	15.00	20.00	3.80	6.60	0.57
			DCP 92-3		
S_0	8.40	18.00	2.40	5.00	0.48
S_1	6.20	14.30	1.80	3.90	0.46
S_2	5.00	10.40	1.28	3.10	0.41
S_3	4.00	7.50	0.84	2.40	0.35
LSD (5%)	A = 0.90	A = 0.70	A = 0.05	A = 0.50	A = 0.20
	T = 0.70	T = 0.50	T = 0.20	T = 4.00	T = 3.00
	I = 1.32	I = 1.40	I = 0.50	I = 6.00	I = 5.00

A = age, T = treatment, I= interaction.
S_0 = control, S_1 = 4 dS m^{-1}, S_2 = 6 dS m^{-1}, S_3 = 8 dS m^{-1}.

which are dependent upon the biological processes and developmental stages of the plant, and also on the type and concentration of salts. The observed decrease in chlorophyll might be attributable to increased chlorophyllase activity. Chlorophyll content is considered an important parameter of salt tolerance in crop plants (James et al., 2002). The small reduction in chlorophyll pigments in CSG 9651 might have been responsible for its higher biomass.

Amino acids accumulation seems to have been directly related to their non-conversion into proteins, whose quantity declined under salt stress (data not shown). Amino acids content in the salt-sensitive cultivar DCP 92-3 increased under the 4 dS m^{-1} treatment and declined under the 6 and 8 dS m^{-1} saline treatments. Salt-induced accumulation of free amino acids has been reported in some legumes by Pessarakli et al. (1989), Fougere et al. (1991), and Soussi et al. (1998), and this accumulation of intracellular amino acids seems to be a prominent physiological response to osmotic stress. The greater accumulation of soluble sugars in salt-tolerant cultivars is likely to be due to less adverse effects of salt stress on amylase activity (Singh et al., 2001). Sugar accumulation in tolerant cultivars is expected to play an important role in conferring tolerance to salt stress (Gorham et al., 1985) and in turn reflects a better balance between anabolic and catabolic processes, which seems to be disturbed to a greater extent in salt-sensitive cultivars. A direct consequence of greater osmotic strength in CSG 9651 was the maintenance of comparatively higher water retaining capacity, as reflected by RWC.

Antagonistic relationships between Na and K uptake, and negative effects of salinity on K uptake have been previously reported in chickpea (Singh and Singh, 1999; Baalbaki et al., 2000). It appears that K^+/Na^+ selectivity may be involved in reducing the damage associated with excessive Na^+ levels in soil. Cusido et al. (1987) reported that decreases in K content are related to increases in the uptake of Na, and that there was antagonism between Na and K. The reduction in K content due to increasing salinity reflects the competition that occurs between Na and K uptake in plants, or the inhibitory effect of Na on K. Baalbaki et al. (2000) suggested that the involvement of 2 physiological mechanisms reduces the impact of salinity, i.e. sodium compartmentalization in the roots and K^+/Na^+ selectivity. It seems that cultivar CSG 9651 under the study conditions showed tolerance because of better osmoregulation due to reduced transport of Na ions from the roots to the shoots. These results indicate that K and Na content were important for salt tolerance. Reduction in yield under salinity may be a cumulative effect of various factors, like a decline in the number of flowers, pod setting, the number of ovules fertilized and nurtured into healthy seeds, and thus the number of seeds per pod and seed weight. Negative effects of salinity on the harvest index seemed to be directly correlated with reduced plant dry mass production in the salt-sensitive cultivar and, hence, inadequate supply of photosynthates to the developing seeds. On the basis of plant biomass and yield analysis under salinity, CSG 9651 was more tolerant to salinity stress than DCP 92-3.

In conclusion, it is apparent that sodium and soluble organic solute accumulation, and higher membrane stability as a result of salinity appeared to play an important role in the acclimation of CSG 9651 to salt stress, suggesting that they could be used as physio-biochemical markers for salt tolerance. These indices might prove useful for improving the salt tolerance of chickpea cultivars. Finally, selection of *Cicer arietinum* cultivars with traits like osmolyte accumulation might be useful in assessing the adaptive responses of *Cicer arietinum* to salinity stress.

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