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Physiological Responses of Chickpea (*Cicer arietinum* L.) Genotypes to Salinity Stress

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A B S T R A C T

Plant growth and development are adversely affected by salinity- a major environmental stress that limits agricultural production. Chickpea (*Cicer arietinum L.*) is sensitive to salinity that affects its yield and there is need to identify the tolerant genotypes. In order to evaluate the effect of soil salinity, a pot experiment with two chickpea genotypes was carried out under screen house conditions. The required amounts of chloride and sulphate salts of Na^{+}, Ca^{+2} and Mg^{+2} were added through NaCl, Na_2SO_4, $CaCl_2$, $MgCl_2$ and $MgSO_4$. Sodium and Ca^{+2} + Mg^{+2} were in the ratio of 1:1 where Ca^{+2} and Mg^{+2} were in the ratio of 1:3 to develop three (2.0, 4.0, 6.0 dS m^{-1}) levels of saline soil before sowing. The control plants were irrigated with distilled water. Sampling was done at 50-60 days after sowing. The water potential (Ψ_w) of leaves, osmotic potential (Ψ_s) of leaves and roots decreased significantly in both the genotypes under different salinity levels. HC-3 showed more negative values of Ψ_w of leaves i.e. from -0.47 to -0.54 MPa as compared to -0.45 to -0.51 MPa in CSG-8962, respectively with increasing salinity level from control to 6.0 dS m^{-1}. Likewise, the Ψ_s of leaves decreased from -0.75 to -1.32 MPa in HC-3 and -0.62 MPa to -1.18 MPa in CSG-8962. With increase in salinity levels, RWC (%) of leaves and roots also declined in both the genotypes. RWC was higher in HC-3 than CSG-8962. The chlorophyll a, chlorophyll b and carotenoid concentration of chickpea genotypes also showed significant reduction under salinity stress as compared to controls. Reduction in photosynthetic pigments was more in CSG-8962 than HC-3. The proline content of leaves increased significantly from 0.573 to 0.904 and 0.565 to 0.782 mg g^{-1} dry weight and the total soluble carbohydrate (TSC) from 17.5 to 24.5 and 16.60 to 20.3 mg g^{-1} dry weight in HC-3 and CSG-8962, respectively with increasing level of salinity from control to 6.0 dS m^{-1}. Salinity levels increased the Cl^{-} concentration in leaves by 93.3 % in HC-3 and 120.1 % in CSG-8962, and SO_4^{2-} by 11.1 % in HC-3 and 19.7 % in CSG-8962 at 6.0 dS m^{-1} salinity levels as compared to their respective controls. The genotype HC-3 had overall lower accumulation of Cl^{-} and SO_4^{2-} than the CSG-8962.More negative values of Ψ_w of leaves, Ψ_s of leaves and roots and better accumulation of osmotically active solutes, i.e. proline and TSC of HC-3, helped in maintaining the higher RWC of these organs than noticed in CSG-8962. The number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds pod^{-1}, test weight and seed yield $plant^{-1}$reduced in both the genotypes with increasing level of salinity from control to 6.0 dS m^{-1}. The reduction is more in CSG-8962 as compared to HC-3. Hence, the mechanism of salt tolerance is relatively better in HC-3 than in CSG-8962 as found from physiological and yield attributes studied and could be used in crop improvement programme of chickpea for salinity tolerance.

Introduction

Chickpea (*Cicer arietinum* Linnaeus), a member of family Fabaceae, is an ancient self-pollinated leguminous crop, diploid annual (2N=16 chromosomes) grown since 7000BC, in different area of the world (Tekeoglu *et al.*, 2000) but its cultivation is mainly concentrated in arid and semi-arid environments such South Asia, West Asia, North Africa, East Africa, Southern Europe, North and South America, and Australia (Arefian *et al.*, 2014; Flowers *et al.*, 2010). In India, Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, Bihar and Jharkhand are major chickpea producing states contributing more than 95% to the total chickpea production. Madhya Pradesh is the single largest producer in the country accounting for over 40% of total production while Rajasthan, Maharashtra, Uttar Pradesh and Andhra Pradesh contribute about 14%, 10%, 9% and 7%, respectively.

The share of Andhra Pradesh and Karnataka has consistently been rising during the past one decade. Further, states like Jharkhand and Chhattisgarh are expanding their area and production of chickpea crop (AICRP, 2014-15). The chickpea seed is a valuable source of carbohydrates and proteins, which together constitute 80% of the total dry seed weight. The crude protein content of chickpea varies from 17% to 24% containing the essential amino acids like tryptophan, methionine and cysteine (Williams and Singh, 1987). Thus, chickpea serves as a main source of dietary protein for more than 80% of the Indian population which is vegetarian in nature. Chickpea acquires importance as it provides food for humans as well as for livestock. Furthermore, chickpea pod covers and seed coats can also be used as fodder. Chickpea nitrogen fixation plays an important role in maintenance of the soil fertility, particularly in the arid and low rainfall area (Roy *et al.*, 2010).

Soil salinity is known as a major inevitable problem, especially in arid and semi-arid regions of the world and affects about 80 million hectare of arable lands (Flowers *et al.*, 2010), 2.95 million hectare in India and 49.2 thousand hectare in Haryana and this area is expanding (Ali, 2009). Despite the high yield potential of chickpea of over 4000 kg per hectare (Singh, 1990). The chickpea suffer losses from salinity both in soil and water (Flowers, 2010). Studying salinity in soil or water is of importance for agriculture because it limits distribution of higher plants in certain natural habitats and induces a wide range of adverse metabolic responses in them.

Salinity causes not only physiological dehydration (water stress) in plants, but also nutrient ion imbalance (Toker *et al.*, 2007). Salinity stress adversely affects several morphological features and physiological processes like reduction in growth, decrease in chlorophyll, ion balance, water status, photo-synthesis, increase in hydrogen peroxide, which causes lipid per oxidation and consequently membrane injury, nodulation and N_2-fixation (Zhu, 2001; Kukreja *et al.*, 2005; Flowers *et al.*, 2010). When plants are subjected to salinity, reactive oxygen species (ROS) are also generated in response to stress conditions which cause chlorophyll degradation; lipid peroxidation and electrolyte leakage are considered to be indicators of oxidative damage. Plants have evolved diverse strategies of acclimatization and avoidance to cope with adverse environment conditions. These include accumulation of compatible osmolytes, antioxidants and enzymes scavenging ROS (Ashraf and Harris, 2004). Proline and carbohydrates are accumulated in plant tissue under saline stress, and these substances subjected to contribute to osmotic adjustment and enhancing salt tolerance.

In recent decades considerable improvements in salinity tolerance have been made in crop species with respect to morphological and physiological characters and traits affecting salinity tolerance, but there is not enough information for chickpea tolerance. Many scientists suggested that selection is more convenient and practical if the plant species possesses distinctive indicators of salt tolerance at whole plant, tissue or cellular levels. This study is designed to determine, aside from growth, the effect of salt stress on physiological and biochemical parameters in chickpea varieties exhibiting differences in salinity tolerance. Comparison of these responses could be useful in identifying differences related to the relative ability of each cultivar to cope with salinity. Results from this study can supply information on the possible potential physiological and biochemical indicators and also could allow deeper insights in to the mechanisms of tolerance to salt-induced stress.

Materials and Methods

Two chickpea (*Cicer arietinum* L.) genotypes CSG-8962 (salt tolerant) and HC-3 (released variety) were raised in pots filled with dune sand [93.3% sand + 3.0 % slit + 3.7 % clay, saturation capacity 25 %, pH 8.2, ECe_2 0.8 dS m^{-1} at 25 ºC, 10.3 mg (N) kg^{-1}, 2.5 mg (P) kg^{-1}, 180 mg (K) kg^{-1}] under screen house conditions in the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125 004, India. The seeds before sowing were surface sterilized and inoculated with effective *Rhizobium culture* (Ca 181). The desired salinity was developed before sowing and maintains four levels (control, 2.0, 4.0 and 6.0 dS m^{-1}) of chloride dominated salinity. The crop was supplied with an equal quality of nitrogen free nutrient solution with at regular interval of 15 d. The chloride (Cl^-) dominated salinity was prepared by using a mixture of different salts such as NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ where Na: Ca + Mg was in the ratio of 1:1 and Ca: Mg in the ratio of 1:3, the Cl: SO_4 ratio was 7:3 on a meq basis. Sampling was done at 50-60 days after sowing (DAS).

Water potential of leaves was measured with the help of pressure chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), between 8 AM to 10 AM. The osmotic potential (Ψs) of leaves and roots was determined with vapour pressure osmometer (Model 5100-B, Wescor, Logan, USA). The relative water content (RWC) of leaves and roots was measured according to Weatherley (1950). These measurements were made between 8 AM to 10 AM (local time) during a sunny day. Chlorophyll and carotenoid contents of leaves were estimated according to the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO). Proline of leaves and roots was estimated spectrophotometrically according to Bates *et al.*, (1973).

Total soluble carbohydrates of leaves and roots were determined with the method of Yemm and Willis (1954). Cl^- content was estimated by an ion analyser (Model L1- 126, Elico, Delhi, India) and expressed as µ moles g^{-1} DW. SO_4^{2-} was estimated by turbidimetric method by Chesnin and Yien (1950). Sodium and potassium contents were estimated using Flame Photometer (Model CL26D, Elico, Delhi, India) and further expressed in Na^+/K^+ ratio. Photochemical efficiency / quantum yield was determined with intact plants in the field with an OS-30P Chlorophyll Flurometer (Opti-Science, Inc., Hudson, USA). Initial (F_0) and maximum (F_m) fluorescence were recorded and variable fluorescence (F_v), derived by subtracting F_o from F_m. Quantum yield/ photochemical efficiency which is F_v/F_m ratios were than calculated. The yield and its attributing characters were recorded at the time of harvesting.

Data were subjected to analysis of variance (ANOVA) using online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar, Haryana, India) and treatment means were compared by the least significant differences (LSD) ($p < 0.05$).

Results and Discussion

The water potential (Ψ_w) of leaves and osmotic potential of leaves and roots decreased significantly in both the genotypes. HC-3 showed more negative values Ψ_w of leaves i.e. from -0.47 to -0.54 MPa as compared to -0.45 to -0.51 MPa in CSG-8962, respectively. The Ψ_s of leaves decreased from -0.75 to -1.32 in HC-3 and -0.62 to -1.18 MPa in CSG-8962 and -0.64 to -0.94 in HC-3 and -0.60 to -0.87 MPa in roots of CSG-8962 with increase in salinity level from control to 6.0 dS m^{-1}. Relative water content (RWC) of leaves decreased significantly from 7.2 to 30.7 % and 4.6 to 21.9 % in CSG-8962 and HC-3 genotypes. Similarly a significant decrease in RWC was observed in both the genotypes of roots i.e. from 5.3 to 29.9 % in CSG-8962 and 2.8 to 21.9 % in HC-3 with increasing salinity levels from control to 6.0 dS m^{-1} (Table 1). The proposed reason for decreasing Ψ_s is that plant adjust to physiological drought conditions caused by salinity to maintain pressure potential (Wright *et al.,* 1997, Kumar *et al.,* 2008). Decline in Ψ_s can be result of either simple passive concentration of solutes due to dehydration or net accumulation of proline and total soluble carbohydrates (TSC). Similar results were reported by Sairam *et al.,* 2002 in wheat genotypes.

Chlorophyll a, chlorophyll b, and carotenoid concentration of chickpea genotypes grown under different levels of salinity are given in figure 1 (a, b, c) The chlorophyll 'a' decrease significantly from 1.40 to 0.870 in HC-3 and 1.35 to 0.603 mg g^{-1} DW in CSG-8962 (Figure 1 a), the chlorophyll 'b' from 0.613 to 0.414 in HC-3 and 0.605 to 0.354 mg g^{-1} DW in CSG-8962 (Figure 1 b), and the carotenoid decrease significantly from 4.50 to 0.314 in HC-3 and 0.424 to 0.225 mg g^{-1} DW in CSG-8962 (Figure 1 c). Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon which led to disorder in synthesizing chlorophyll and appearing chlorosis in plant. Overall the genotype HC-3 showed that less reduction in photosynthetic pigments compared to CSG-8962. Similarly in mungbean seedling, chlorophyll a, b and carotenoid contents were greatly reduced under salt stress (Zayed and Zeid, 1997-98). The quantum yield (Fv/Fm) of leaves decreased from 0.712 to 0.593 and 0.726 to 0.599 in CSG-8962 and HC-3, respectively increasing salinity levels from control to 6.0 dS m^{-1} (Figure 1 d). Hall and Rao (1999) reported that analysis of fluorescence characteristics such as quantum yield reflects the properties of the chlorophyll molecules and their interaction with the external environment and also with associated physiological processes.

The proline content of leaves was increased i.e. from 0.565 to 0.782 and 0.573 to 0.904 mg g^{-1} dry weight at 50-60 DAS (Figure 2 a) in the genotypes CSG-8962 and HC-3, respectively. Similarly, the proline content of roots was found to be increased significantly in both the genotypes from 0.090 to 0.265 and 0.098 to 0.305 mg g^{-1}DW in the genotypes CSG-8962 and HC-3, respectively (Figure 2 a). Accumulation of proline was more in roots than leaves as later were directly in contact with salt impregnated soil sphere. A rapid accumulation of proline under salt stress has been observed in mungbean crop (Singh *et al.,* 1994) and chickpea (Kumar *et al.,* 2008).

Table.1 Changes in water potential Ψ_w (-MPa), osmotic potential Ψ_s (-MPa) and relative water content (RWC %) of chickpea genotypes under different salinity levels

Parameters	Genotypes	Salinity levels(dS m^{-1})									
		0	2	4	6	M					
		Leaves									
Ψ_w	HC-3	0.47	0.48	0.50	0.54	0.49					
	CSG 8962	0.45	0.46	0.47	0.51	0.47					
	Mean	0.46	0.47	0.48	0.53						
	CD at 5 %	Genotype = 0.01; Salinity = 0.02; G x S = NS									
Ψ_s		Leaves					Roots				
		0	2	4	6	M	0	2	4	6	M
	HC-3	0.75	1.03	1.15	1.32	1.06	0.64	0.74	0.86	0.94	0.80
	CSG 8962	0.62	0.93	1.02	1.18	0.94	0.60	0.63	0.80	0.97	0.73
	Mean	0.68	0.98	1.09	1.25						
	CD at 5 %	Genotype = 0.02; Salinity = 0.03; G x S = NS					Genotype = 0.14; Salinity = 0.20; G x S = 0.28				
RWC	HC-3	92.61	88.37	79.65	72.26	83.22	95.19	92.50	85.43	74.29	86.85
	CSG 8962	88.72	82.38	71.56	61.46	76.03	93.41	88.47	79.28	65.41	81.64
	Mean	90.66	85.37	75.60	66.86		94.30	90.48	82.36	69.85	
	CD at 5 %	Genotype = 0.27; Salinity = 0.39; Gx S = 0.55					Genotype = 1.57; Salinity = 2.23; G x S = 3.15				

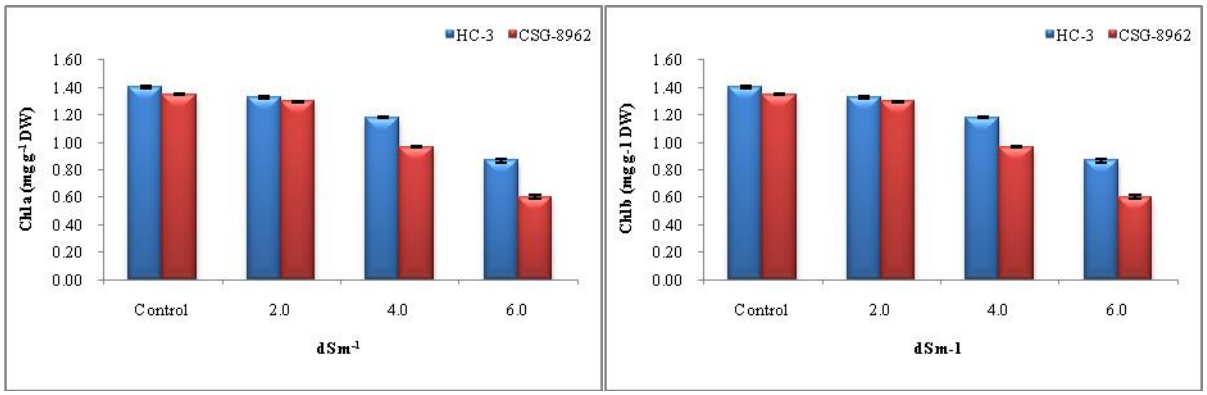
Table.2 Changes in Cl^- content (mg g^{-1}DW), SO_4^{2-} content (mg g^{-1}DW) and Na^+/K^+ ratio of chickpea genotypes under different salinity levels

Parameters	Genotypes	Salinity levels(dS m^{-1})									
		0	2	4	6	M	0	2	4	6	M
		Leaves					Roots				
Cl^-	HC-3	0.600	0.617	0.867	1.160	0.811	0.643	0.627	0.927	1.237	0.865
	CSG 8962	0.610	0.640	0.917	1.343	0.878	0.603	0.703	1.103	1.557	0.992
	Mean	0.606	0.626	0.891	1.250		0.623	0.665	1.028	1.37	
	CD at 5 %	Genotype = 0.024; Salinity = 0.034; G x S = 0.048					Genotype = 0.022; Salinity = 0.031; G x S = 0.043				
SO_4^{2-}	HC-3	0.573	0.610	0.620	0.637	0.610	0.597	0.607	0.667	0.673	0.636
	CSG 8962	0.587	0.627	0.643	0.703	0.640	0.623	0.630	0.667	0.697	0.654
	Mean	0.580	0.618	0.632	0.670		0.600	0.610	0.660	0.680	
	CD at 5 %	Genotype = 0.015; Salinity = 0.021; G x S = NS					Genotype = 0.016; Salinity = 0.023; G x S = NS				
Na^+/K^+ ratio	HC-3	0.143	0.171	0.221	0.288	0.206	0.204	0.251	0.372	0.539	0.341
	CSG 8962	0.166	0.213	0.295	0.364	0.259	0.208	0.282	0.388	0.624	0.376
	Mean	0.155	0.192	0.258	0.326		0.206	0.267	.380	0.582	
	CD at 5 %	Genotype = 0.006; Salinity = 0.009; G x S = 0.012					Genotype = 0.008; Salinity = 0.011; G x S = 0.016				

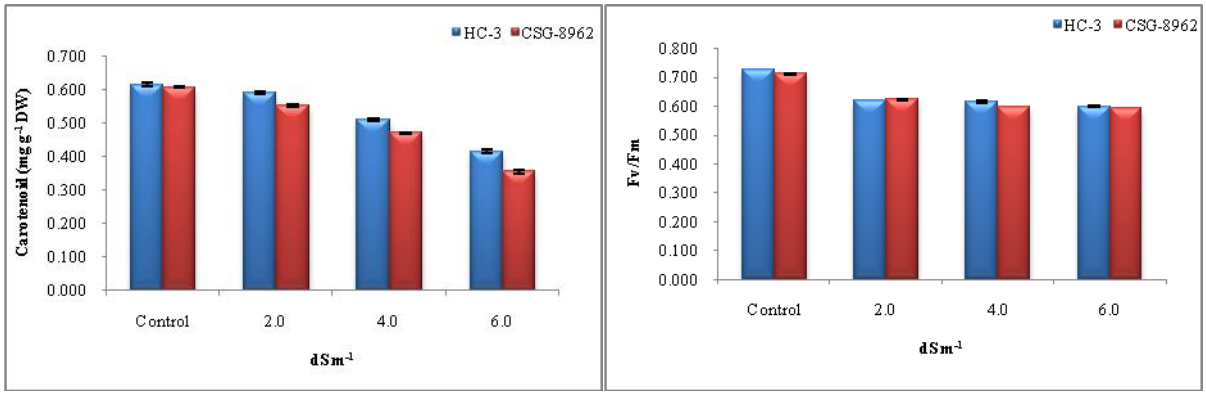
Table.3 Changes in yield and its attributes of chickpea genotypes under different salinity levels

Parameters	Genotypes	Salinity levels(dS m^{-1})				
		0	**2**	**4**	**6**	**M**
Branches $plant^{-1}$	HC-3	9.00	8.33	8.00	6.33	7.91
	CSG-8962	8.00	7.00	6.6	5.00	6.91
	Mean	8.50	8.16	7.33	5.66	
	CD at 5 %	Genotype = 0.30; Salinity = 0.43; G x S = NS				
Pods $plant^{-1}$	HC-3	13.66	13.33	11.33	8.66	11.75
	CSG-8962	12.66	11.33	8.33	7.66	10.00
	Mean	13.16	12.33	9.83	8.16	
	CD at 5 %	Genotype = 0.50; Salinity = 0.71; G x S = 1.00				
Seeds pod^{-1}	HC-3	1.66	1.33	1.33	1.33	1.41
	CSG-8962	1.66	1.30	1.00	1.00	1.24
	Mean	1.66	1.31	1.16	1.16	
	CD at 5 %	Genotype = 0.04; Salinity = 0.05; G x S = 0.08				
100 seed weight (g)	HC-3	29.36	24.72	15.75	10.97	20.20
	CSG-8962	13.68	10.82	8.83	7.47	
	Mean	21.52	17.77	12.29	9.22	
	CD at 5 %	Genotype = 0.51; Salinity = 0.72; G x S = 1.02				
Seed yield $plant^{-1}$(g)	HC-3	26.00	25.00	23.00	19.00	23.25
	CSG-8962	25.00	23.00	22.33	16.00	21.58
	Mean	25.50	24.00	22.66	17.50	
	CD at 5 %	Genotype = 0.59; Salinity = 0.84; G x S = 1.19				

Fig.1 Changes in chlorophyll a (a), chlorophyll b (b), carotenoid content (c) and quantum yield (d) of chickpea genotypes under different salinity levels

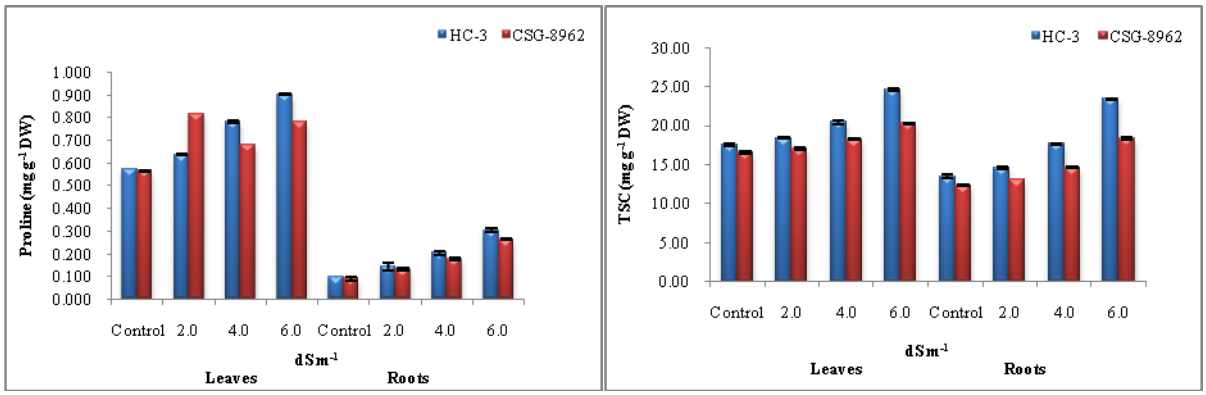


(a) (b)



(c) (d)

Fig.2 Changes in proline content (a) and total soluble carbohydrates (b) of chickpea genotypes under different salinity levels



(a) (b)

The total soluble carbohydrates of leaves increased from 16.60 to 20.24 and 17.55 to 24.56 mg g^{-1} DW (Figure 2b) in the genotypes CSG-8962 and HC-3 and in roots from 12.9 to 18.4 and 13.4 to 23.4 in CSG-8962 at 50-60 DAS respectively.

Similarly Tawfic (2008) also reported an increase in total soluble carbohydrates in cowpea plants grown under salt stress.

The Cl^- content in leaves increased120.1 % in CSG-8962 and 93.3 % in HC-3 genotype and in roots by 158.2 % and 92.3 % at 6.0 dS m^{-1}salinity level in CSG-8962 and HC-3, respectively (Table 2). The SO_4^{2-} content was increased by 19.7 % in leaves of salinised plants of CSG-8962 as compared by 11.1 % in HC-3 than their corresponding controls and similarly in roots the SO_4^{2-} content increased from 1.1 to 11.8 and 1.6 to 12.7 % in the genotypes CSG-8962 and HC-3, respectively.

Similar result was found that sulphate content also decreased with progressive increase in salinity level in leaves and stem but increased in roots of sea black horn (Chen *et al.*, 2009).

Number of branches $plant^{-1}$ reduced to 37.5 % and 29.6 % in the genotypes CSG-8962 and HC-3, respectively, at 6.0 dS m^{-1} salinity level.

The number of pods $plant^{-1}$ reduced to 39.5 % and 36.6 % in the genotypes CSG-8962 and HC-3, respectively. The percent reduction in number of seeds pod^{-1} was 39.7 % in CSG-8962 and 19.8 % in HC-3. The percent

reduction in test weight was 7.4 % in CSG-8962 and 10.9 % in HC-3 at 6.0 dS m^{-1}.

The percent reduction in seed yield $plant^{-1}$was 27.3, 43.8 and 58.0 % and 19.0, 31.5 and 52.0% in the genotypes CSG-8962 and HC-3, respectively at 2.0, 4.0 and 6.0 dS m^{-1} salinity level with respect to their control (Table 3).

Turner *et al.,* (2013) also observed that saline treatment (40mM NaCl) significantly decreased the seed yield in chickpea genotypes and genotypic variation for salinity tolerance exists in chickpea.

HC-3 showed comparative better perform than CSG-8962 on the basis of various physiological traits related to plant water relations, chlorophyll, osmolyte accumulation, ionic distribution and yield attributes under saline conditions.

Abbreviations

dS m^{-1} – DeciSiemens per metre, DAS – Days after sowing, DW - Dry weight, MPa - Mega Pascal, RWC - Relative water content, TSC-Total soluble carbohydrates, Ψ_w - Water potential, Ψ_s - Osmotic potential

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