

## BIOCHEMICAL CHANGES IN RESPONSE TO SALINITY IN CHICKPEA (*CICER ARIETINUM* L.) DURING EARLY STAGES OF SEEDLING GROWTH

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### ABSTRACT

Salinity is a serious abiotic stress, causing oxidative stress. Various biochemical parameters in chickpea genotypes were considered under varied NaCl concentrations (0, 8 and 12 dS.m<sup>-1</sup>). This experiment was done as factorial arrangement (genotype × salt concentration × time) in a completely randomized design. Samples were collected at 21 and 28-day old seedlings (28-DOS). The results revealed that increasing salt concentration resulted in higher levels for malondialdehyde content; among genotypes, MCC806 with 2.2 and MCC760 with 0.7 had the highest and lowest amount, respectively. Proline and protein contents were significantly higher in MCC544 by 27-fold increase (for proline) and 30% (for protein) relative to control in 28 DAS at 12 dS.m<sup>-1</sup> of salt. The leaf soluble carbohydrates also increased significantly in MCC544 and MCC760 compared with the others. The minimum decline of electrolyte leakages (6%) was belonged to MCC760 while MCC806 genotypes showed the highest decrease rate (more than 20%). Total leaf chlorophyll content decreased in all genotypes during the stress. However, morphological damages in MCC544 and MCC760 genotypes were less in 28-DOS at 8 and 12 dS.m<sup>-1</sup> NaCl, respectively. Overall, proline and leaf soluble carbohydrates were more consistent with salt tolerance responses of the genotypes, and 2 weeks after stress initiation (28-DOS) could be a critical stage for screening the genotypes.

**Key words:** Chickpea, Proline, Salinity, Total chlorophyll, Total soluble protein.

### INTRODUCTION

Chickpea is the third most important pulse crop in the world in terms of total production which is mostly grown in semi-arid regions such as South Asia, West Asia, North Africa, East Africa, Southern Europe, North and South America, and Australia (Roy *et al.*, 2010). It is cultivated in more than 50 countries with over 11 million hectares, and its total annual world production is around 8.4 million tons (FAOSTAT, 2011). It is cultivated in more than 50 countries with over 11 million hectares, and its total annual world production is around 8.4 million tons (FAOSTAT, 2011). Chickpea is a valuable source of protein, carbohydrate, fiber and many essential vitamins and minerals. Chickpea nitrogen fixation plays an important role in maintenance of the soil fertility, particularly in the arid and low rainfall areas (Roy *et al.*, 2010).

It is estimated that around 20% of total land in the world and nearly half of all irrigated land are adversely influenced by salinity stress (Silva and Gerós, 2009). Salinity causes not only physiological dehydration (water stress) in plants, but nutrient ion imbalance (Toker *et al.*, 2007). Under saline conditions, reactive oxygen species (ROS) are commonly generated and accumulated by which oxidative damage occurs in bio-molecules such as lipids and proteins, resulting in cell death later in the process (Molassiotis *et al.*, 2006; Shad *et al.*, 2013). Soil

salinity is known as a major inevitable constrain, especially in arid and semi-arid regions of the world where these regions are the main cultivation areas of chickpea (Khan *et al.*, 2013).

Despite chickpea sensitivity to salinity, particularly at the early stages of growth and development, there has been a considerable variation observed among various genotypes in which the most susceptible ones fail to grow in just 25 mM NaCl but tolerant genotypes survives up to a maximum of 100 mM NaCl in hydroponics (Flowers *et al.*, 2009). In addition, the higher levels of salt concentrations in the soil due to its accumulation and drying the soil towards the end of the growing season, both lead to 8 to 10% yield losses globally. However, it is suggested that selection of tolerant genotypes would be an appropriate strategy to alleviate the adverse implications of salinity (Flowers *et al.*, 2009).

A considerable variation for salinity resistance has been reported among chickpea genotypes in some studies. Serraj *et al.* (2004) screened 234 chickpea genotypes grown in a Vertisol treated with 80 mM NaCl solution. They reported a 60% reduction in biomass at 40 day after sowing and identified resistant genotypes based on salinity susceptibility index (SSI) and shoot biomass. Similar study was achieved by Kafi *et al.*, (2011) in which resistant genotypes was determined under 8 and 12 dS.m<sup>-1</sup> NaCl concentrations 4 weeks after plant

establishment in hydroponic system through evaluation of biochemical parameters such as soluble carbohydrates, proline and photosynthetic pigments.

The current study was aimed to evaluate chickpea responses to salinity with the following particular objectives: (i) to compare the chickpea genotypes in terms of their variation in reaction to varied concentrations of salt stress (various NaCl levels in the soil); (ii) to determine the best biochemical parameter (s) and its reliability as a marker for fast assessment and screening of the genotypes in reaction to salinity condition; (iii) to create an optimal physiological framework for further exploration of salt tolerance mechanism among contrastive genotypes using molecular approaches such as proteomics.

## MATERIALS AND METHODS

Seeds of chickpea genotypes were provided by Research Center for Plant Sciences, Ferdowsi University of Mashhad, Iran. Based on our previous salinity study (Arefian *et al.* in press) and others reports (Kafi *et al.*, 2011) we used MCC544 and MCC760 as tolerant and MCC361, MCC773 and MCC806 as susceptible genotypes.

Seeds were surface sterilized three times with 3% (w/v) sodium hypochlorite for 1 min, followed by 70% ethanol for 30 s and rinsed with sterile water five times and germinated in petri dishes for 48 hr, prior to sowing. Two chickpea seedlings were grown in each pot with one-liter capacity, filled with a mixture of field soil and sand (2:1, w/w) and kept in controlled conditions ( $25\pm 2^\circ\text{C}$ ,  $50\pm 5\%$  relative humidity and 16-hr photoperiod with light intensity of  $270 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and then treated with salty water after 2 weeks for 14 consecutive days.

The effect of different concentrations of NaCl (0, 8 and  $12 \text{ dS}\cdot\text{m}^{-1}$ ) on various biochemical parameters were measured among the genotypes as a factorial experiment in a completely randomized design with 3 replicates in two growth stages of early seedling growth (21-day old seedlings) and flowering initiation (28-day old seedlings).

Proline was extracted from 0.2 g leaf tissues homogenized in 4 ml 3% aqueous sulfosalicylic acid using the method developed by Bates *et al.*, (1973). Briefly, after centrifugation at 10000 rpm, 2ml of supernatant was mixed with 2 ml of ninhydrin and 2 ml of glacial acetic acid, and then boiled at  $100^\circ\text{C}$  for 1 hour. The reaction mixture was extracted by 4ml toluene and its absorbance was measured at 590 nm. Final proline concentration was calculated by the standard curve and following formula:

$$\text{Proline } (\mu\text{mol}\cdot\text{gFW}^{-1}) = \left( \frac{\mu\text{g prolin}}{\text{ml}} \times \frac{\text{ml toluen}}{115.5 \left( \frac{\mu\text{g}}{\mu\text{mol}} \right)} \right) \frac{\text{gr sample}}{5}$$

Total soluble proteins were determined through some modifications in Lowry *et al.*, (1951) method. In brief, 0.1M potassium phosphate buffer was used for extraction, and then the concentration of the proteins was calculated by BSA standard curve. The membrane lipid peroxidation was determined by the method from Heath and Packer (1968), in terms of malondialdehyde (MDA) production. Thus, 0.2g fresh leaf tissue was ground in 5ml 0.1% Trichloro acetic acid (TCA) and centrifuged at 10000 rpm. The supernatant was mixed well with 20% TCA, containing 0.5% thiobarbituric acid in 1:4 (v/v) ratio, and boiled at  $90^\circ\text{C}$  for 30 min. Oxidized MDA was calculated according to the following formula:

$$\text{MDA } (\mu\text{mol} / \text{gFW}) = A_{532-600} / 1.55 \times 10^{-5} \text{ M cm}^{-1} \times b$$

The total chlorophyll, calculated by adding chlorophyll a and b with following formula in fresh leaf samples, was extracted in 80% acetone and estimated by the method of Lichtenthaler and Buschmann (2001).

$$c_a (\mu\text{g/ml}) = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$c_b (\mu\text{g/ml}) = 21.50 A_{646.8} - 5.10 A_{663.2}$$

The carbohydrates were measured using the procedure of Dubois *et al.* (1956). Briefly, dried powder of 100 mg leaf DW was vortexed with 80% ethanol. After removing the supernatant along with extra sediments by adding 5% zinc sulphate and barium hydroxide 0.3 normal, it was mixed with phenol (2:1 (v/v)) and then with 1.5N  $\text{H}_2\text{SO}_4$  (5:1 (v/v)). The absorbance was read at 490 nm, using spectrophotometer (OPTIMA, sp-3000 plus) after 45 min.

Membrane stability index (MSI) based on electrolyte leakage was assayed by estimating the ion leaching from leaves into distilled water (Premachandra *et al.*, 1990). The leaves were transferred to 10 mL distilled water in two sets. The first set was kept at  $40^\circ\text{C}$  for 30 min and then its conductivity (C1) recorded using a conductivity meter. The second set was kept at  $100^\circ\text{C}$  for 10 min and its conductivity (C2) also recorded and finally MSI was calculated through  $(C1/C2) \times 100$ .

Data were subjected to analysis of variance (ANOVA) and significant differences among means were calculated by Duncan's multiple range test ( $p \leq 0.05$ ). The percentage and relative data were normalized by converting to arc sinus and square root. All calculations were performed in SAS version 6.12 and jump version 4.0.4 softwares, and the figures plotted by Excel 2013.

## RESULTS AND DISCUSSION

In the present study, proline content of leaves significantly increased ( $p \leq 0.05$ ) with the increase of NaCl concentrations in all genotypes (Fig. 1). This might contribute to osmotic adjustment under salt stress. Increased proline level is due to protein breakdown (Evan Ibrahim, 2012). Once carbohydrate is available in the leaf

cells or tissues, proline would engage with proteins. If a portion of the accumulated proline were still present when carbohydrates in the leaves is depleted by translocation and respiration processes, then proline would be synthesized through oxidation along with normal protein synthesis (Kafi *et al.*, 2011). High salinity treatment resulted in 27 and 17 fold higher proline content compared to the control in MCC544 and MCC760, respectively. At 8 dS.m<sup>-1</sup> concentration, the highest proline level was observed for MCC544 in both samplings 21 and 28 days with 9 and 20 fold increase; these values were significantly higher than those of the other genotypes. It seems that defense response of MCC760 to the high level of stress (12 dS.m<sup>-1</sup>) was stronger than others; so this genotype showed a higher level of tolerance to salinity at this level. At the highest NaCl concentration in both samplings, MCC806 showed significantly the lowest proline accumulation than those

of the other genotypes, especially compared with MCC544 with 4.7 and 2.9 times less proline in 21-day old seedlings.

Proline is a particular osmolyte in plants, increasing rapidly under reduced water levels and assist the plants to preserve cell turgor (Bidabadi *et al.*, 2012). This osmolyte is a compatible solute, which can be considered as protective response in terms of osmotic adjustment (OA) in abiotic stress condition (Ali *et al.*, 2007 ; Mahajan and Tuteja, 2005). The increase of proline upon salt stress in tolerant genotypes was consistent with the findings of other studies (Najaphy *et al.*, 2010 ; Singh, 2004). Based on this parameter, MCC544 and MCC760 can be considered as tolerant while MCC806 the most susceptible one. The more delay in proline accumulation was observed in susceptible genotypes.

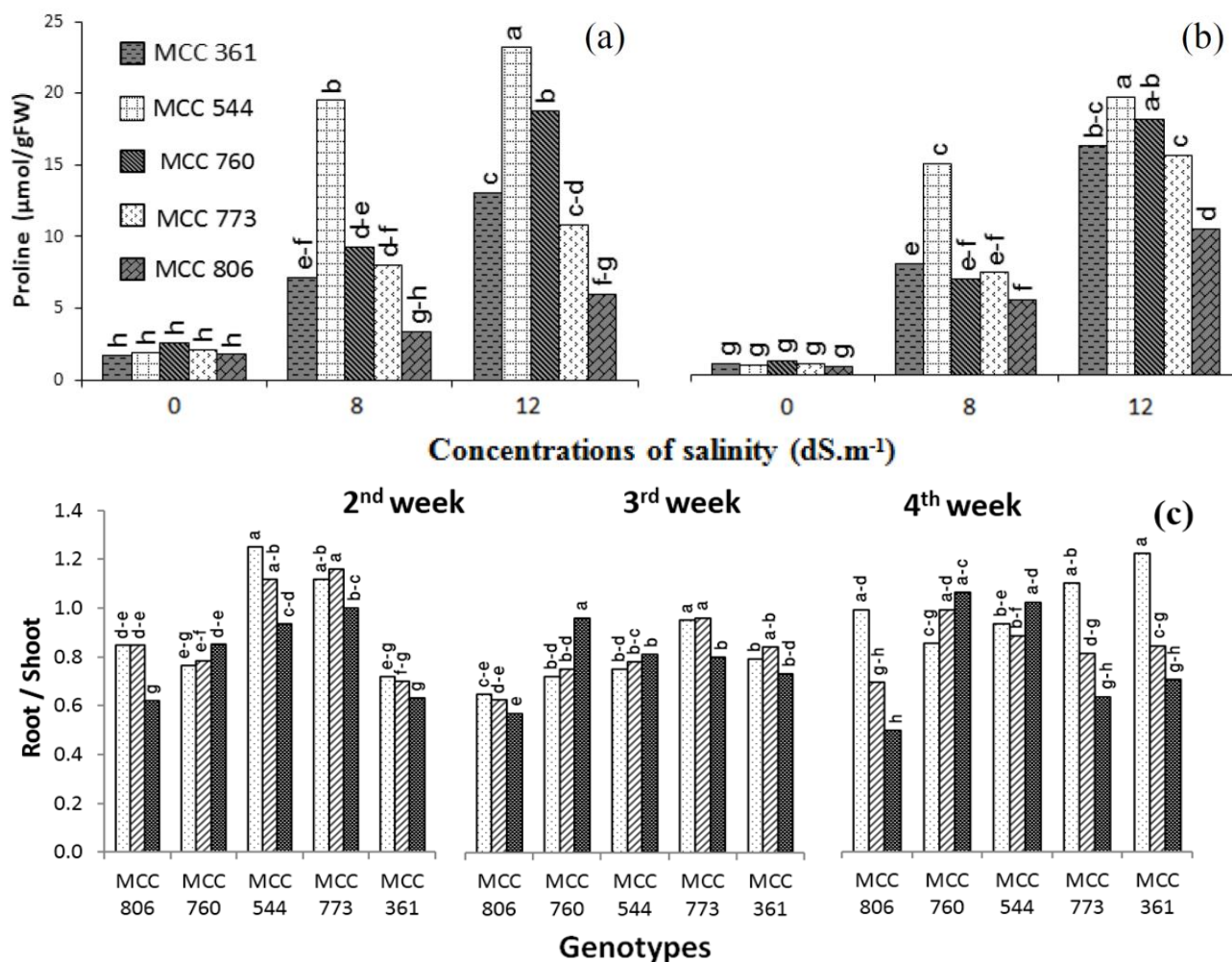
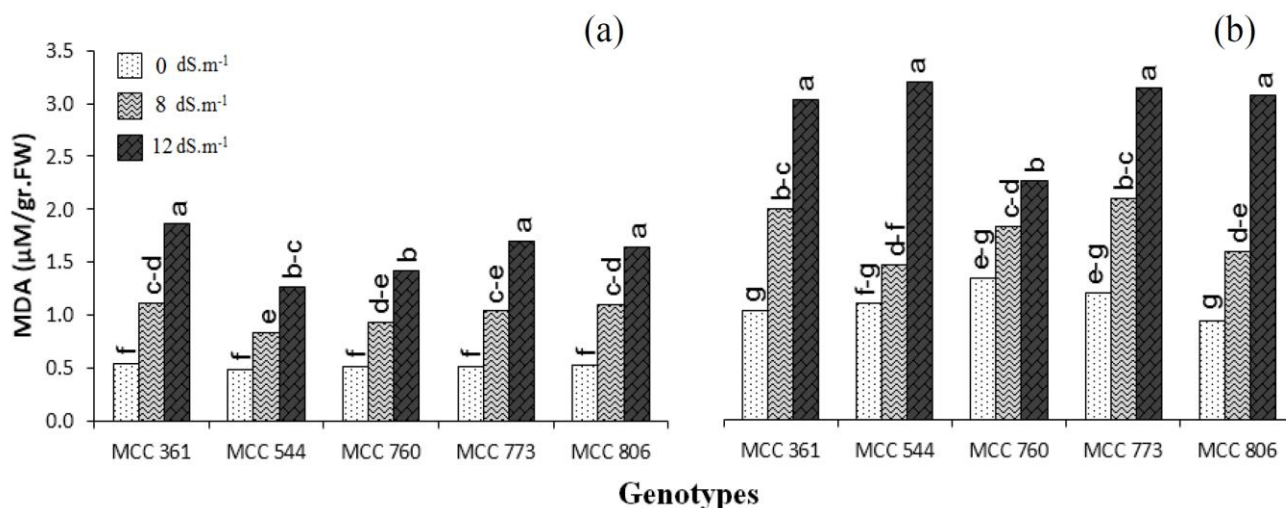


Figure 1: Proline content changes among genotypes under various salinity concentrations in (a) 21 and (b) 28 DAS and (c) Root dry weight to Shoot Dry Weight ratio. Means in columns with at least one letter in common in the range are not significantly different ( $p \leq 0.05$ ).

ROS are strong oxidizing species that create oxidative damages to bioactive molecules including lipids and proteins and these processes finally result in cell death. MDA, a lipid peroxidation product, has been used as an appropriate biomarker to evaluate the free radicals levels in the living cells (Molassiotis *et al.*, 2006). In the current study, MDA content of all genotypes had a progressive increase with rising salinity levels over time (Fig. 2). Among all genotypes, MCC760 showed

relatively less increase in MDA content. At high NaCl application to 28-day seedlings, the lowest and highest MDA changes observed for MCC760 and MCC806 genotypes (with 0.7 and 2.2 fold increase as compared to control treatment), respectively (Fig. 2). The responses of genotypes were different in 21-day seedlings in which MCC544 and MCC760 had the lowest increase in MDA content (1.6 fold) but the highest (2.3 fold increase) for MCC806 and MCC361 genotypes.

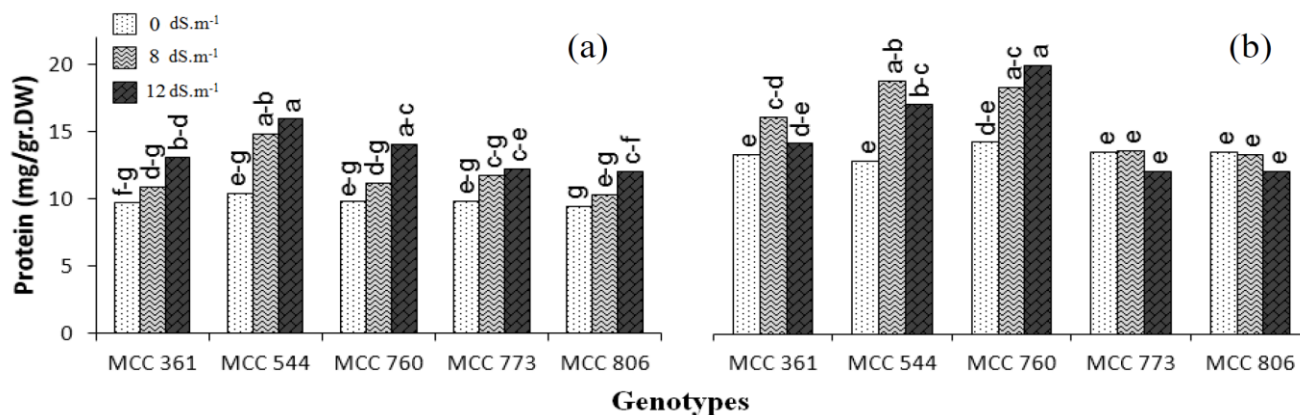


**Figure 2: Salinity impact on malondialdehyde (MDA) content of chickpea genotypes, in (a) 21 and (b) 28 DAS. Means in columns with at least one letter in common in the range are not significantly different ( $p \leq 0.05$ ).**

The increase in MDA content under salinity and drought stresses especially in susceptible genotypes was in agreement with the findings of Bian and Jiang (2009) in chickpea. Increase in MDA content may reduce the ability to scavenge oxygen radical species accumulation which might be an explanation for the higher membrane damage in leaf tissue (Bandoğlu *et al.*, 2004). This possible mechanism is later supported by higher electrolyte leakage (decreases of membrane stability index) under salt stress. According to this parameter, it seems that the older seedlings (28-day) are better stage for genotypes to be compared.

Salinity has a dual influence in relation to the protein pattern in the plants. It reduces the total protein content (Delgado *et al.*, 1993), and also commences the synthesis of other specific proteins necessary for tolerating the effect of salinity through engaging ABA (Chen and Plant, 1999). The pattern of total protein changes was evident among genotypes studied in reaction to NaCl treatments (Fig. 3). The impact of higher

concentration of salinity (8 and 12 ds.m<sup>-1</sup>) was more prominent on MCC544 and MCC760 genotypes in which the values significantly raised. In 28-day old seedlings, MCC760 accumulated not only the highest protein content (20 mg/gr.DW), but also had the highest increase (40%) over the control. A slight decrease in protein content for susceptible genotypes was recorded, especially in MCC806. Insufficient increase in proline and protein content of these genotypes may be due to the degradation of some biomolecules such as enzymes (Arora *et al.*, 2002). This might be an indication of their inability to maintain cell turgor under salinity condition (Ashraf and Tufail, 1995). Overall, significant differences of leaf total soluble proteins were observed in 28-day old seedlings. It was also revealed that tolerant genotypes (such as MCC760) had more proline and protein content than other susceptible ones. An increase in protein content upon salt stress has been reported in different tolerant plant species (Najaphy *et al.*, 2010).

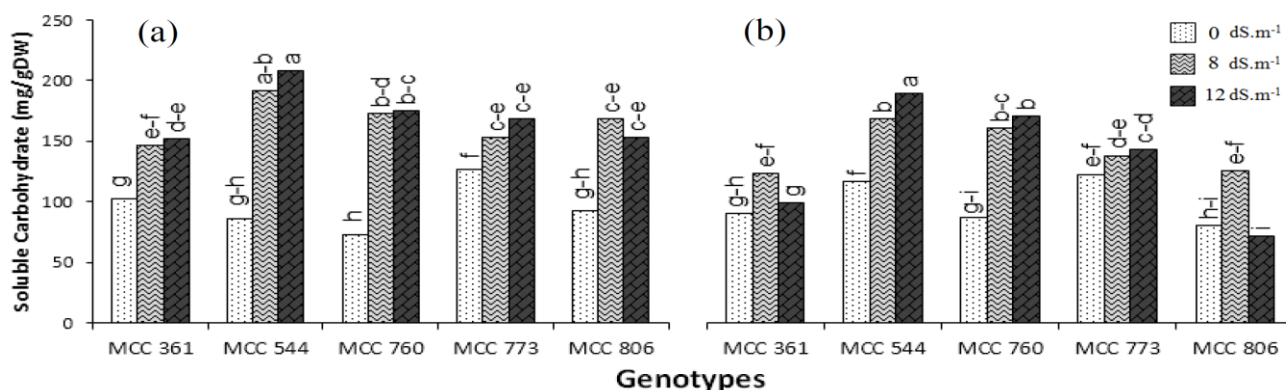


**Figure 3.** Effect of NaCl on protein content of genotypes in (a) 21 and (b) 28 DAS. Means in columns with at least one letter in common in the range are not significantly different ( $p \leq 0.05$ ).

Among various organic osmotica, sugars form up to 50% of the total osmotic potential in glycophytes plants subjected to saline conditions (Parvaiz and Satyawati, 2008). The accumulation of carbohydrates including sugars and starch, facilitating the osmotic adjustment, has been largely reported in response to salinity or drought (Mahajan and Tuteja, 2005). The content of soluble carbohydrates significantly changed with increasing the salinity level (Fig. 4). The mixed responses were observed among genotypes. For instance, tolerant genotypes (MCC760 and MCC544) had the highest carbohydrates accumulation under higher salinity level, especially in 21-day old seedlings, so that salt treatments caused 1.32 and 1.47 fold increase of carbohydrates content in 28-day old seedlings, and 0.9 and 0.53 in 28-day old seedlings, and 0.9 and 0.6 in 28-day old seedlings for these two genotypes, respectively. Salt-induced reduction in soluble carbohydrate content was seen in susceptible genotypes especially MCC806 at higher level of stress in 28-day old seedlings.

The soluble carbohydrates accumulation in tolerant genotypes seems to play an essential role in conferring tolerance characteristic to salt condition. This may refer to the better balance between anabolic and

catabolic processes in which susceptible genotypes can be disturbed to a greater extent. Carbohydrates function as metabolic signals during stress condition and act a critical role in osmoprotection, osmotic balance, carbon preservation, membrane stability and radical scavenging (Parvaiz and Satyawati, 2008). More significantly, high differences observed in 28-day old seedlings and this might be a critical time for comparison in the current study. Munns (1993) reported that in early exposure of tolerant wheat genotypes to salt stress, soluble carbohydrates increased due to converting sucrose to monosaccharides. Increase of leaf soluble carbohydrates of chickpea in reaction to salinity has been reported by Kafi *et al.*, (2011). One possible reason presented was reduction or interruption in the transfer of carbohydrates from shoot to root of the plant to maintain osmotic balance between cytoplasm and vacuole. As observed in this study, Ashraf and Tufail (1995) found that although sugar content increased considerably through increasing salt level, tolerant lines of sunflower had mostly greater soluble sugars than the salt sensitive ones. In the similar context, there are many reports, indicating that the soluble carbohydrates content increase in response to salt stress (Meloni *et al.*, 2004).



**Figure 4.** The average leaf soluble carbohydrates of genotypes in (a) 21 and (b) 28 DAS. Means in columns with at least one letter in common in the range are not significantly different ( $p \leq 0.05$ ).

In addition to MDA content, electrolyte leakage measurement is another commonly used criterion to assess the extent of oxidative stress and level of membrane stability, which is associated with leakage of solutes from the cells (Bandeoglu *et al.*, 2004). MCC760 displayed maximum maintenance of cell membrane integrity in which only 6% decrease of membrane

stability index (MSI) occurred relative to the control under salt condition (Fig. 5). Meanwhile, MCC806 showed an obvious decline (20%) of MSI in response to salt. In agreement with this result, MSI decrease has been mentioned in susceptible genotypes of chickpea under stress (Bhushan *et al.*, 2011).

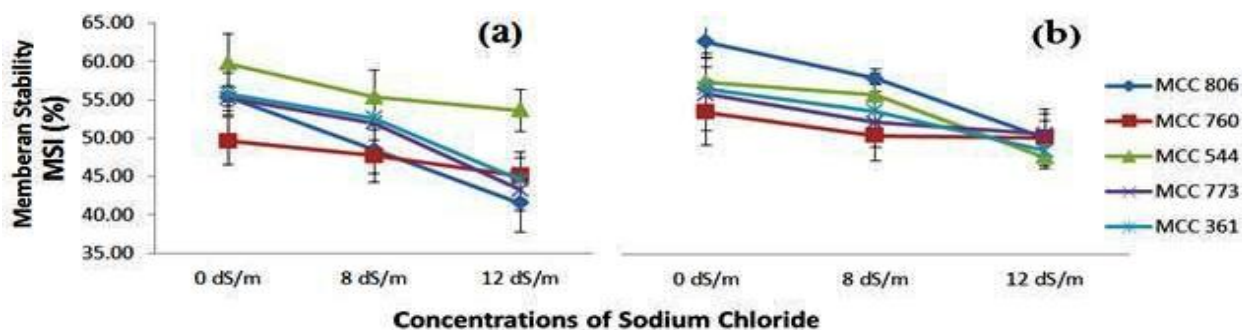


Figure 5: Membrane stability coefficient trends among chickpea genotypes under varying salinity concentrations in (a) 21 and (b) 28 DAS.

Salinity stress can be recognized in the leaves by decrease of MSI and chlorophyll content. The adverse effects of salinity on pigments content has been reported in chickpea (Mudgal *et al.*, 2009). This measurement has been used in genotype selection for salinity tolerance and sensitivity. The status of total chlorophyll content was measured to give an insight into photosynthetic capabilities in all studied genotypes. In consistent with other studies (Yadav *et al.*, 2011), a higher chlorophyll degradation was observed in salt-sensitive chickpea genotypes in the current study. The rate of decline and loss of total chlorophyll contents increased with higher NaCl treatments and it was found to be significantly less up to 24% in tolerant genotype, MCC760, in 28-day old

seedlings (Fig. 6). The reduction in chlorophyll and other pigments content due to salinity may reduce carbon fixation that eventually supply energy and substrates for metabolic pathways. This finally may cause reduction in plant growth and development (Yadav *et al.*, 2011).

In various experiments, pigments contents showed a mixed reaction, depending on exposure time and salt concentration. The reduction of total chlorophyll amounts in chickpea upon salt stress was widely reported (Beltagi, 2008). The potential cause may be due to increasing of destructive enzymes called chlorophyllase. Pigments system reduction is attributed to weakening of protein-pigment-lipid complex induction or elevated chlorophyllase enzyme activity (Rahdari *et al.*, 2012).

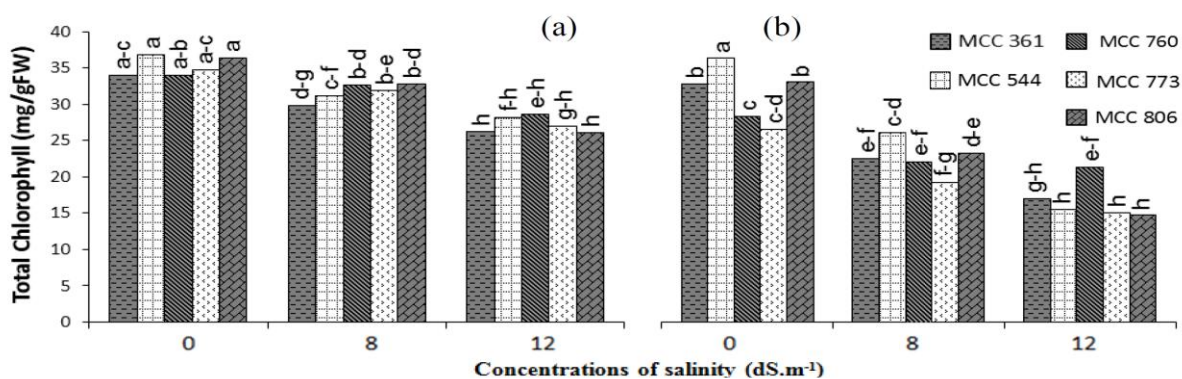


Figure 6. Total Chlorophyll content under different salinity concentrations in (a) 21 and 28 DAS. Means in columns with at least one letter in common in the range are not significantly different ( $p \leq 0.05$ ).

To study mechanism of tolerance, correlations of biochemical parameters was compared between tolerant and susceptible genotypes after initial screening of genotypes in table 1. There was a strong and positive

correlation in tolerant genotypes between proline and carbohydrates (0.80\*\*) as well as proline and protein (0.60\*\*), but no significant correlation in susceptible ones (Table 1). In tolerant genotypes, proteins and

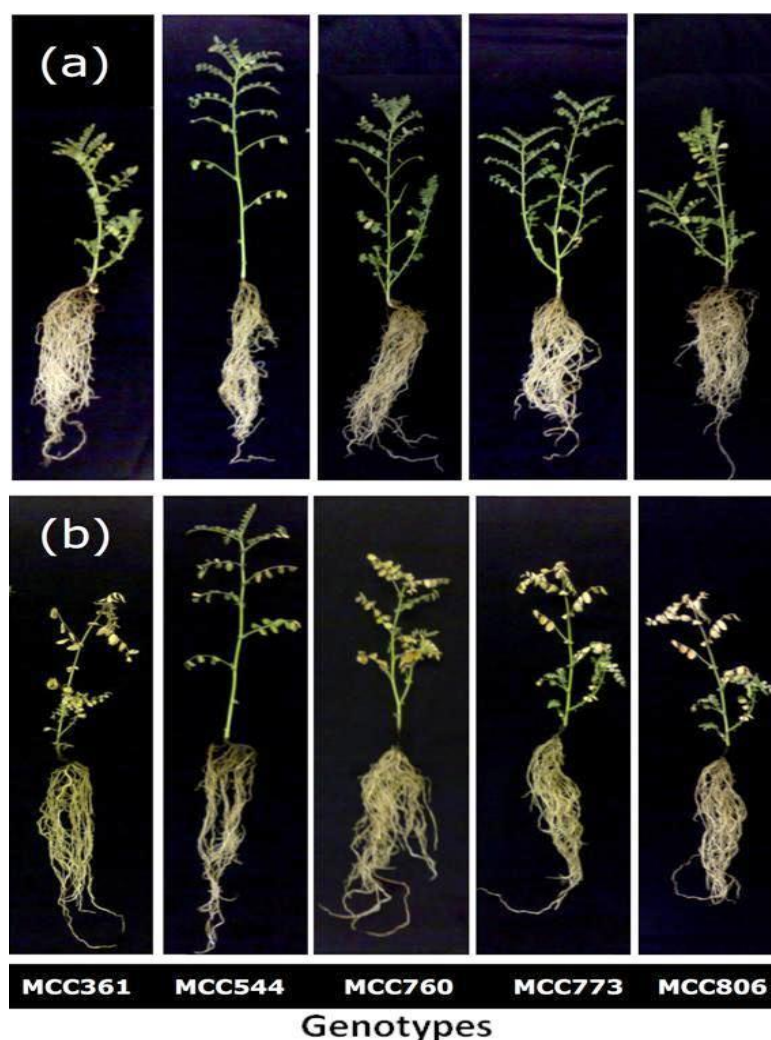
carbohydrates accumulation possibly resulted in membrane stability (0.26\* and 0.61\*\* correlations values), meanwhile membrane damage in susceptible genotypes might be due to decrease of necessary proteins and carbohydrates since the correlation values were negative. Chlorophyll degradation and MDA accumulation in leaves of tolerant genotypes might be due to the increase

in root-shoot ratio (0.51\*\* and -0.37\*\*), a proposed tolerance mechanism (Kalefetoglu Macar *et al.*, 2009 ; Mensah *et al.*, 2009). In the current study, carbohydrate accumulation had a negatively relation with shoot dry mater (0.23\*). This may be due to less photosynthesis rate (Kafi *et al.*, 2011).

**Table 1: Correlation values between each pair of biochemical parameters in tolerant (bold data) and susceptible (un-bold data) genotypes of chickpea at 28 DAS seedlings under salt stress condition.**

	<i>1. Proline</i>	<i>2. MDA</i>	<i>3. Protein</i>	<i>4. Carbo</i>	<i>5. MSI</i>	<i>6. Chl</i>	<i>7. Ro/Sh</i>
<i>1. Proline</i>	1	<b>0.63**</b>	<b>0.60**</b>	<b>0.80**</b>	- 0.21*	- 0.58**	<b>0.33*</b>
<i>2. MDA</i>	0.97**	1	<b>0.26*</b>	<b>0.57**</b>	- 0.46**	- 0.81**	<b>0.51**</b>
<i>3. Protein</i>	0.01	0.001	1	<b>0.61**</b>	<b>0.26*</b>	- 0.46**	<b>0.02</b>
<i>4. Carbo</i>	0.07	0.13	0.001	1	- 0.25*	- 0.52**	<b>0.23*</b>
<i>5. MSI</i>	- 0.29*	- 0.34*	- 0.14	- 0.09	1	<b>0.52**</b>	<b>0.01</b>
<i>6. Chl</i>	- 0.78**	- 0.85**	0.001	- 0.29*	0.40**	1	- <b>0.37**</b>
<i>7. Ro/Sh</i>	- 0.13	- 0.07	- 0.23*	0.23*	0.16	0.002	1

\*and \*\* are significant data at 0.05 and 0.01 levels, respectively.



**Figure 7: Effects of NaCl stress on 28 DAS of chickpea genotypes under two concentrations (a) 0 dS.m<sup>-1</sup>, (b) 12 dS.m<sup>-1</sup>.**

Overall, according to results of this study, although proline, leaf soluble carbohydrates and total chlorophyll were introduced as the best biochemical criteria for screening of chickpea genotypes in response to NaCl stress, the other parameters studied could be useful criteria as extra supporting evidence in determining the tolerance of MCC760 and susceptibility of MCC806 genotypes. Furthermore, the data showed that the best time for screening the genotypes seems to be 28-day old seedlings in which the most significant differences occurred among genotypes based on biochemical parameter assessments.

## REFERENCES

- Arefian, M., S. R. Vessal, A. B. Bagheri and A. Ganejali (2014). Trend of changes for morpho-physiological characteristics of chickpea (*Cicer arietinum* L.) under salinity stress (NaCl) condition. Iranian J. Pulses Res., In press.
- Arora, A., R. Sairam and G. Srivastava (2002). Oxidative stress and antioxidative system in plants. Curr. Sci., 82: 1227-1238.
- Ashraf, M. and M. Tufail (1995). Variation in salinity tolerance in sunflower (*Helianthus annuum* L.). J. Agron. Crop Sci., 174: 351-362.
- Bandoğlu, E., F. Eyidoğan, M. Yücel and H. A. Öktem (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regul., 42: 69-77.
- Bates, L., R. Waldren and I. Teare (1973). Rapid determination of free proline for water-stress studies. Plant Soil., 39: 205-207.
- Beltagi, M.S., (2008). Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chickpea (*Cicer arietinum* L.). Afr. J. Plant Sci., 2: 118-123.
- Bhushan, D., D. K. Jaiswal, D. Ray, D. Basu, A. Datta, S. Chakraborty and N. Chakraborty (2011). Dehydration-responsive reversible and irreversible changes in the extracellular matrix: comparative proteomics of chickpea genotypes with contrasting tolerance. J. Proteome Res., 10: 2027-46.
- Bian, S. and Y. Jiang (2009). Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci. Hortic., 120: 264-270.
- Bidabadi, S. S., S. Meon, Z. Wahab, S. Subramaniam and M. Mahmood (2012). In vitro selection and characterization of water stress tolerant lines among ethyl methanesulphonate (EMS) induced variants of banana (*Musa* spp., with AAA genome). Aust. J. Crop Sci., 6: 567-575.
- Chen, C.C. and A.L. Plant (1999). Salt-induced protein synthesis in tomato roots: the role of ABA. J. Exp. Bot., 50: 677-687.
- Delgado, M., J. Garrido, F. Ligerio and C. Lluch (1993). Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride stress. Physiol. Plant., 89: 824-829.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. t. Rebers and F. Smith (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.
- Evan Ibrahim, A. J., (2012). Effect of water stress on carbohydrate metabolism during *Pisum sativum* *Pisum sativum* seedlings growth. Euphrates J. Agric. Sci., 4: 1-12.
- Flowers, T.J., P. M. Gaur, C. L. L. Gowda, L. Krishnamurthy, S. Samineni, K. H. M. Siddique, N. C. Turner, V. Vadez, R. K. Varshney and T. D. Colmer (2009). Salt sensitivity in chickpea. Plant Cell Environ., 33: 490-509.
- Heath, R.L. and L. Packer (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125(1), 189-198.
- Kafi, M., A. Bagheri, J. Nabati, M. Zare Mehrjerdi and A. Masomi (2011). Effect of salinity on some physiological variables of 11 chickpea genotypes under hydroponic conditions. J. Sci. Technol. Greenhouse Cult., 1: 55-70.
- Khan, S., J. Iqbal and M. Saeed (2013). Comparative study of grain yield and biochemical traits of different rice varieties grown under saline and normal conditions. J. Anim. Plant Sci. 23: 575-588.
- Lichtenthaler, H. K. and C. Buschmann (2001). Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Curr. Protoc. Food Analyt. Chem. Published Online.
- Lowry, O.H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Mahajan, S. and N. Tuteja (2005). Cold, salinity and drought stresses: an overview. Arch. Biochem. Biophys., 444: 139-158.
- Meloni, D.A., M.R. Gulotta, C. A. Martínez and M. A. Oliva (2004). The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Braz. J. Plant Physiol., 16: 39-46.
- Molassiotis, A., T. Sotiropoulos, G. Tanou, G. Diamantidis and I. Therios (2006). Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the

- apple rootstock EM 9 (*Malus domestica* Borkh). *Environ. Exp. Bot.*, 56: 54-62.
- Mudgal, V., N. Madaan, A. Mudgal and S. Mishra (2009). Changes in growth and metabolic profile of Chickpea under salt stress. *J. Appl. Biosci.*, 23: 1436-1446.
- Munns, R. (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell Environ.*, 16: 15-24.
- Najafy, A., N. N. Khamssi, A. Mostafaie and H. Mirzaee (2010). Effect of progressive water deficit stress on proline accumulation and protein profiles of leaves in chickpea. *Afr. J. Biotechnol.*, 9: 7033-7036.
- Parvaiz, A. and S. Satyawati (2008). Salt stress and phyto-biochemical responses of plants-a review. *Plant Soil Environ*, 54: 89-99.
- Premachandra, G., H. Saneoka and S. Ogata (1990). Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soybean. *J. Agric. Sci.*, 115: 63-66.
- Rahdari, P., S. Tavakoli and S. M. Hosseini (2012). Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleracea* L.) Leaves. *J. Stress Physiol. Biochem.*, 8: 182-193.
- Roy, F., J. Boye and B. Simpson (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Res. Int.*, 43: 432-442.
- Shad, M. A., H. Nawaz, T. Rehman and N. Ikram (2013). Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of *Cichorium intybus* L.: a comparative study. *J. Anim. Plant Sci.*, 23: 1060-1066.
- Silva, P. and H. Gerós (2009). Regulation by salt of vacuolar H<sup>+</sup>-ATPase and H<sup>+</sup>-pyrophosphatase activities and Na<sup>+</sup>/H<sup>+</sup> exchange. *Plant Signal. Behav*, 4: 718-726.
- Toker, C., C. Lluch, N. Tejera, R. Serraj and K. Siddique (2007). Abiotic stresses. In: Chickpea Breeding and Management (S.S. Yadav, R.J. Redden, W. Chen and B. Sharma eds). CABI, Oxfordshire, UK, pp. 474-496.
- Yadav, S., M. Irfan, A. Ahmad and S. Hayat (2011). Causes of salinity and plant manifestations to salt stress: a review. *J. Environ. Biol.*, 32: 667-689.