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Original Research Article

Biochemical Changes and SDS-PAGE Analyses of Chickpea (*Cicer arietinum* L.) Genotypes in Response to Salinity During the Early Stages of Seedling Growth

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ABSTRACT

Salinity is one of the most serious abiotic stresses for plants, causing other subsequent consequences such as oxidative stress, which eventually leads to cell death. Measured various biochemical parameters in chickpea genotypes were performed under various NaCl concentrations (0, 8 and 12 dS.m^{-1}) in controlled condition at 21 and 28-day after sowing (DAS). After determination of tolerant (MCC544) and susceptible (MCC806) genotypes and also the best differential salt concentration, SDS-PAGE was used to compare protein profiling in these two genotypes in 3 time points with four replicates. Proline and protein contents were significantly higher in MCC544 as much as 27 fold (for proline) and 30% (for protein) increase over control in 28 DAS at 12 dS.m^{-1} of salt. The leaf soluble carbohydrates increased significantly in MCC544 and MCC760, compared with others. The minimum decline of electrolyte leakages (6%) and malondialdehyde (MDA) content was belonged to MCC760 while MCC806 genotype showed the highest decrease rate (more than 20%). Total leaf chlorophyll content decreased in all genotypes. More strong and positive correlations between parameters was recorded in tolerant genotypes which resulted in membrane and osmotic balance. Analyses of SDS-PAGE revealed that more rapid accumulation and/or less degradation of proteins occurred in higher molecular weight proteins. Moreover, the response of genotypes through protein changes before 96 h stress might be a possible reason for salinity tolerance in this condition.

Key Words: Chickpea, Proline, Protein, SDS-PAGE, Salinity

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). Chickpea is a valuable source of protein, carbohydrate, fiber and many essential vitamins and minerals (Roy *et al.*, 2010). Chickpea nitrogen fixation plays an important role in maintenance of the soil fertility, particularly in the arid and low rainfall areas (Varshney *et al.*, 2009).

Salinity is one of the most serious abiotic stresses in agriculture worldwide which is estimated that some 20% of total land in the world and nearly half of all irrigated land are adversely influenced by this stress (Silva and Gerós, 2009). Salinity causes not only physiological dehydration (water stress) in plants, but also 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). Soil salinity is known as a major inevitable problem, especially in arid and semi-arid regions of the world, where these regions are the main cultivation areas of chickpea (Flowers *et al.*, 2009).

Despite chickpea sensitivity to salinity, particularly at the early stages of growth and development, there has been reported 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 concentration 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 (Flowers *et al.*, 2009). However, it is suggested that selection of tolerant genotypes would be an appropriate strategy to alleviate the adverse implications of salinity (Hasegawa *et al.*, 2000).

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A considerable variation for salinity resistance has been reported among chickpea genotypes in some studies, but a few of them, compared correlations of parameter in tolerant and susceptible which was performed in present study. Moreover, protein profiling by SDS-PAGE revealed novel information on resistance in salt stress condition. However, 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 has been achieved by Kafi *et al.* (2011) in which resistant genotypes was determined under 8 and 12 $dS.m^{-1}$ 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 achieved to evaluate chickpea responses to salinity through biochemical parameters and protein profiling 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 level 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 saline condition; (iii) to investigate changes in profile of proteins in tolerant genotype to possibly explain the mechanism of salinity tolerance using SDS-PAGE.

MATERIAL AND METHODS

Plant material and experiment design

Seeds of chickpea genotypes were provided by Research Center for Plant Sciences, Ferdowsi University of Mashhad, Iran. Based on our previous salinity study and others reports (Kafi *et al.*, 2011), we used MCC544 and MCC760 as putative tolerant and MCC361, MCC773 and MCC806 as putative 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 for five times and germinated in petri dishes for 48 hr prior to sowing. Two chickpea seedlings were grown in each one-liter pot , filled with a mixture of field soil and sand (2:1, w/w) and kept in controlled conditions (25±2°C, 50±5% relative humidity and 16-hr photoperiod with light intensity of 270 µmol m^{-2} s^{-1}), and then treated with saline water after 2 weeks for 14 consecutive days.

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

Biochemical parameters measurement

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, then boiled at 100°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 equation:

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

Total soluble proteins were determined through some modifications in Lowry *et al*. (1951) method. In breif, 0.1M potassium phosphate buffer was used for extraction, 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°C for 30 min. Oxidized MDA was calculated according to the following equation:

$$\text{MDA}(\mu mol/gFW) = A_{532\text{-}600}/1.55\times10^{-5}\,Mcm^{-1}\times b$$

For total chlorophyll a and b content, extractions from fresh leaf samples were performed in 80% acetone and estimated by the method of Lichtenthaler and Buschmann (2001) using following equation.

$$c_a\,(\mu g/ml) = 12.25\,A_{663.2} - 2.79\,A_{646.8}$$
$$c_b\,(\mu g/ml) = 21.50\,A_{646.8} - 5.10\,A_{663.2}$$

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 and 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 H2SO4 (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°C for 30 min and then its conductivity (C1) recorded using a conductivity meter. The second set was kept at 100°C for 10 min and its conductivity (C2) also recorded and finally MSI was calculated through (C1/C2) ×100.

Total protein extraction, purification and SDS-PAGE

In order to SDS-PAGE analyses of contrasting salt tolerant responses genotypes, total protein was extracted using developed method by Goggin *et al*., (2011). Briefly, 2.5 g of leaveas were ground to a powder in liquid nitrogen, then placed in a centrifuge tube with two volumes of extraction buffer containing 8 M urea, 2% (v/v) Triton X-100, 5 mM DTT. After 20 min incubation on ice with gentle rocking, the tubes were centrifuged at 12000 g for 10 min.

For purification, extracted proteins were precipitated in chilled methanol (-80°C) and incubated overnight at -80°C, then centrifuged for 30 min at 10000 g. The pellet was allowed to air-dry, re-suspended in minimal IEF buffer which contained 8 M urea, 2% [w/v] CHAPS, 60 mM DTT, 2% [v/v] IPG buffer, for 10 min with gentle rocking, and centrifuged at 12000 g for 30 min again.

Protein concentration of samples were determined using Bradford assay and crystalline BSA (Bradford, 1976). To obtain standard curve, absorbance of BSA concentrations (0, 5, 10, 15, 20 and 25 µg/µl) was recorded by OPTIMA spectrophotometer (sp-3000) at 595 nm. To a series of concentrations, 200 µL of Bio-Rad Dye Reagent Concentrate was added to each microtube, then mixed well and incubated at room temperature for 15 min.

SDS-PAGE electrophoresis was performed by 12% acrylamide separating gel and 4% acrylamide stacking gel with 0.5 mm spacers. The separating gel contained 6.68 mL of MQ water, 5 mL of 1.5 M Tris-HCl, pH 8.8, 200 µL of 10% (w/v) sodium dodecylsulphate (SDS), 8 mL of 30% acrylamide (monomer:crosslinker ratio 37.5:1), 10 µL of TEMED and 100 µL of 10% (w/v) ammonium persulphate. The stacking gel consisted of 1.2 mL of MQ water, 0.5 mL of 0.5 M Tris-HCl, pH 6.8, 20 µL of 10% (w/v) SDS, 267 µL of 30% acrylamide, 2 µL of TEMED and 10 µL of 10% (w/v) ammonium persulphate.

10 µg of protein was mixed in a microtube with 20% 5X SDS-PAGE conraining 1/20 volume of β-mercaptoethanol in a final volume of 10 µL. After incubation at 95°C for 5 min and a brief cooling on ice, 10 µL was loaded per lane and electrophoresed at 20 mA for 5 hours with 1X SDS-PAGE running buffer (100 mM glycine, 25 mM Tris and 0.1% (w/v) SDS).

The gels, were stained with Coomassie Brilliant Blue R-250 solution [40% (v/v) methanol, 10% (v/v) acetic acid and 0.25% (w/v) Coomassie Brilliant Blue R-250] and rocked at room temperature for 30 min. These

gels were destained [in 40% (v/v) methanol, 10% (v/v) acetic acid and 3% (v/v) glycerol] at room temperature. Gel image was digitalized at 600 dpi using a GS-800 Calibrated Densitometer (Bio-Rad).

Data analyses

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 9.2 and jump version 4.0.4 softwares, and the figures plotted by Excel 2013. QuantityOne (Version 4.6.3) and GelQuant Pro (Version 12.1) softwares were used to analysethe images and create master gel images from four replicate gels for each individual genotypes at each time points.

RESULTS AND DISCUSSION

Biochemical experiment

In the present study, proline content of leaves had a significant increase ($p \leq 0.05$) with the increase of NaCl concentrations in all genotypes but decreased during time (Fig. 1). Increased proline level could be due to protein breakdown (Evan Ibrahim, 2012). High salinity treatment resulted in accumulation in 27 and 17 fold higher proline content compared to the control in MCC544 and MCC760, respectively. At 8 $dS.m^{-1}$ treatment, MCC544 recorded the highest proline level in both samplings. At the highest NaCl concentration, MCC806 had the lowest proline accumulation significantly.

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.

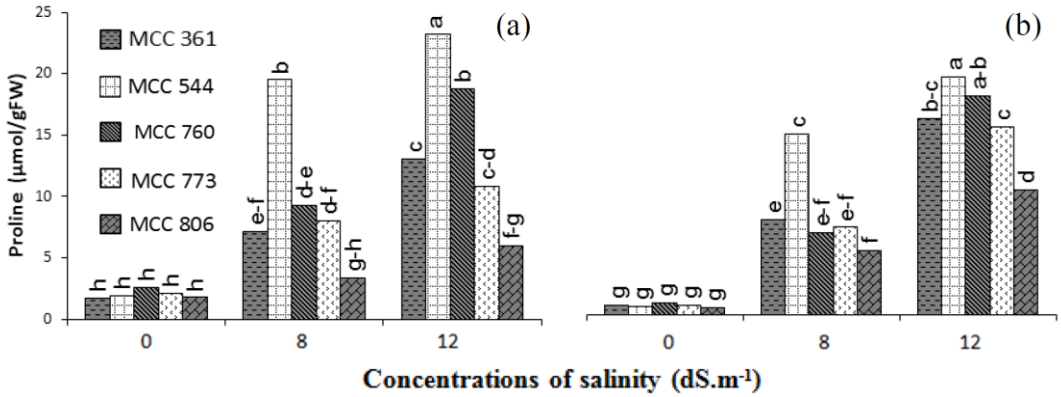

Figure 1. Changes of proline content (µmol/g.FW) in chickpea genotypes under three salinity concentrations (0, 8 and 12 $dS.m^{-1}$), in 21 DAS seedlings (a) and 28 DAS seedlings (b). Means in columns with at least one letter in common in the range are not significantly different ($p \leq 0.05$).

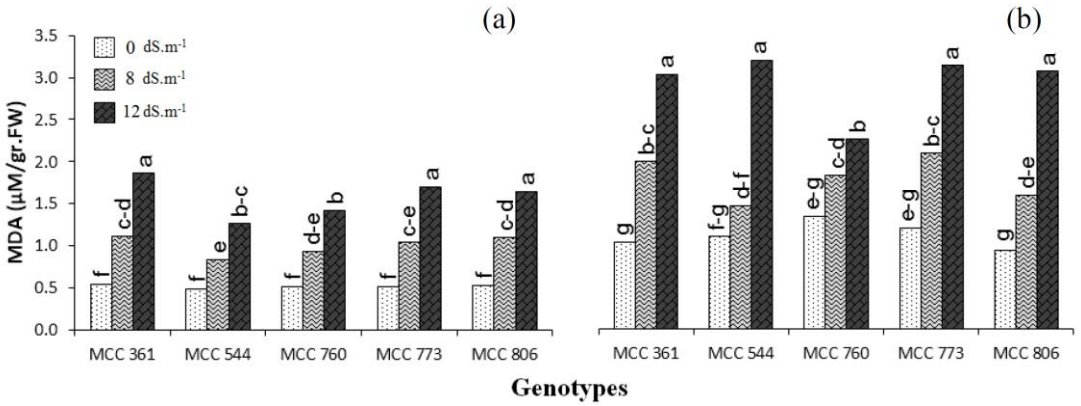

Figure 2. Impact of salinity (NaCl) on malondialdehyde (MDA) content (µmol/g.FW) in chickpea genotypes, in 21 DAS seedlings (a) and 28 DAS seedlings (b). Means in columns with at least one letter in common in the range are not significantly different ($p \leq 0.05$).

MDA, a lipid peroxidation product, has been used as an appropriate biomarker to evaluate the free radicals levels in the living cells and membrane damage (Molassiotis *et al.*, 2006). In current study, MDA content of all genotypes had a progressive increase with rising salinity levels over time (Fig. 2 and 7). At high NaCl application in 28 DAS, the lowest MDA observed in MCC760 genotype (with 0.7 fold increase as compared to control treatment) (Fig. 2). The responses of genotypes were different in 21 DAS in which MCC544 and MCC760 had the lowest increase in MDA content (1.6 fold) while the highest (2.3 fold increase) was recorded in MCC806 and MCC361 genotypes (Fig. 2).

The increase in MDA content under salinity and drought stresses especially in susceptible genotypes, was in agreement with the findings of various study in chickpea (Bian and Jiang, 2009), wheat (Fu and Huang, 2001) and maize (Moussa and Abdel-Aziz, 2008). Increased MDA is result of ability reduction to scavenge ROS (Bandeoğlu *et al.* 2004). This possible mechanism was later supported by higher electrolyte leakage (decrees of membrane stability index [MSI]) confirming our findings. As noted in table 1, increase in MDA resulted in significant decrease in MSI.

Total protein raised during stress times, especially in tolerant genotypes. In 28 DAS 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 was revealed in MCC806. Insufficient increase in proline and protein content of these genotypes may be due to the degradation of some biomoleculars such as enzymes (Arora *et al.*, 2002 ؛ Karagözler *et al.*, 2008 ؛ Nunes *et al.*, 2008). This might be an indication of their inability to maintain cell turgor under saline condition (Ashraf and Harris, 2004). Salinity has a dual influence on protein pattern in the plants. It reduces the total protein content (Delgado *et al.*, 1993) and commences the synthesis of other specific proteins (Chen and Plant, 1999) necessary for tolerating the effect of salinity through engaging ABA (Zeevaart and Creelman, 1988). Confirmed with our findings, enhance in protein content upon salt stress is reported in different tolerant plant species (Amini and Ehsanpour, 2005 ؛ Ashraf and Harris, 2005 ؛ Najaphy *et al.*, 2010). Due to the importance of proteins as functional molecules in controlling cellular processes, a more accurate investigation of protein pattern changes between contrastive genotypes was also achieved using SDS-PAGE with another precise extraction method at a critical stress time points.

The content of soluble carbohydrates significantly changed with increasing the salinity level (Fig. 4). MCC760 and MCC544 had the highest carbohydrates accumulation under higher salinity level, especially in 21 DAS seedlings, so that salt treatments caused 1.32 and 1.47 fold increase of carbohydrates content in 21 DAS, and 0.9 and 0.53 in 28 DAS seedlings in these two genotypes, respectively. 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). Salt-induced reduction in soluble carbohydrate content was observed especially in MCC806. More accumulation of carbohydrates in MCC760 and MCC544 probably including sugars and starch

,which facilitate osmotic adjustment (OA) (Mahajan and Tuteja, 2005 ؛ Parida *et al.*, 2002), function as metabolic signals (Aghaleh *et al.*, 2009) and has a critical role in osmoprotection, carbon preservation, membrane stability and radical scavenging (Parvaiz and Satyawati, 2008). Increased soluble carbohydrates could be due to converting sucrose to monosaccharaides (Munns, 1993). Another possible reason presented by Kafi *et al.*, is reduction or interruption in transferring of carbohydrates from shoot to root of seedlings in order to maintain osmotic balance between cytoplasm and vacuole. Confirmed with our data, there are other reports indicating that the soluble carbohydrates content increase in response to salt stress especially in tolerant varieties (Meloni *et al.*, 2004 ؛ Murakeözy *et al.*, 2003).

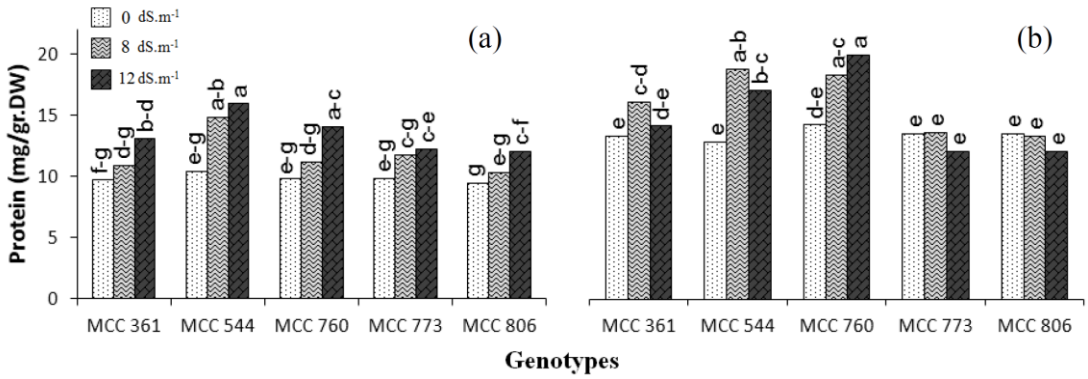

Figure 3. Effect of salinity (NaCl) on protein content (mg/g.DW) of chickpea genotypes in 21 DAS seedlings (a) and 28 DAS seedlings (b). Means in columns with at least one letter in common in the range are not significantly different ($p \leq 0.05$).

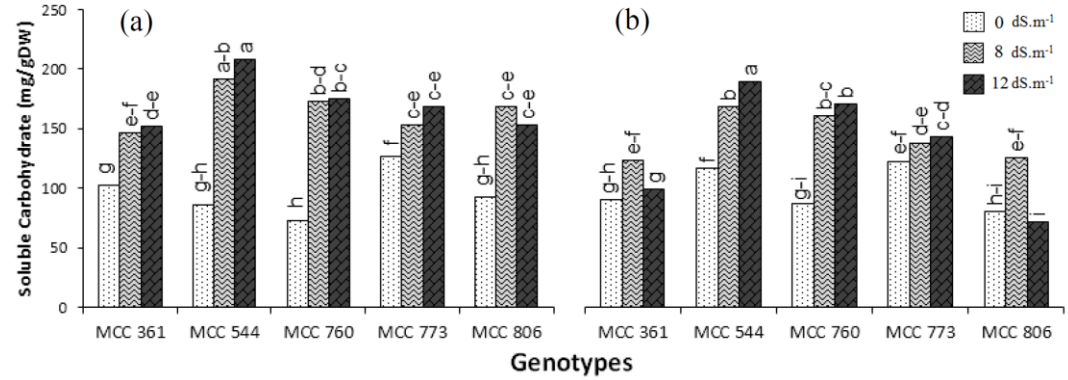

Figure 4. The average leaf soluble carbohydrates (mg/g.DW) of chickpea genotypes in 21 DAS seedlings (a) and 28 DAS seedlings (b). Means in columns with at least one letter in common in the range are not significantly different ($p \leq 0.05$).

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%) in MSI in response to salt. 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, associated with leakage of solutes from the cells (Bandeoğlu *et al.* 2004). In agreement with this result, MSI decrease has been shown in susceptible genotypes of chickpea under stress (Bhushan *et al.*, 2011 ؛ Chohan and Raina, 2011).

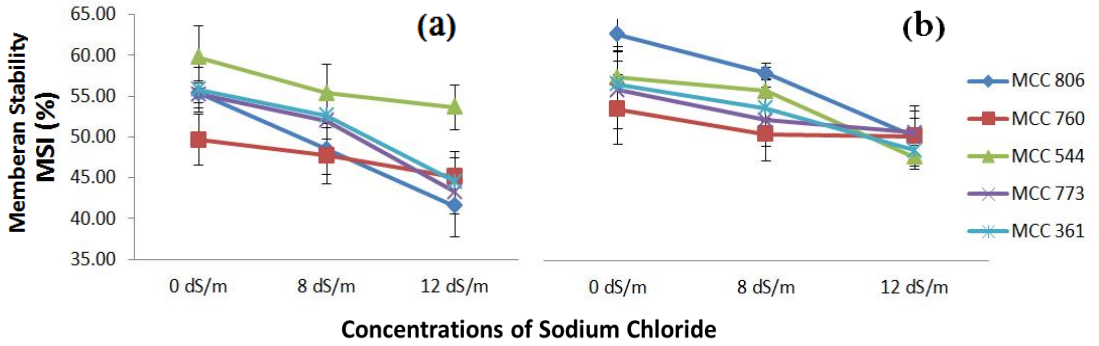

Figure 5. The decline of membrane stability coefficient in chickpea genotypes under three salinity concentrations (0, 8 and 12 dS.m^{-1}), in 21 DAS seedlings (a) and 28 DAS seedlings (b).

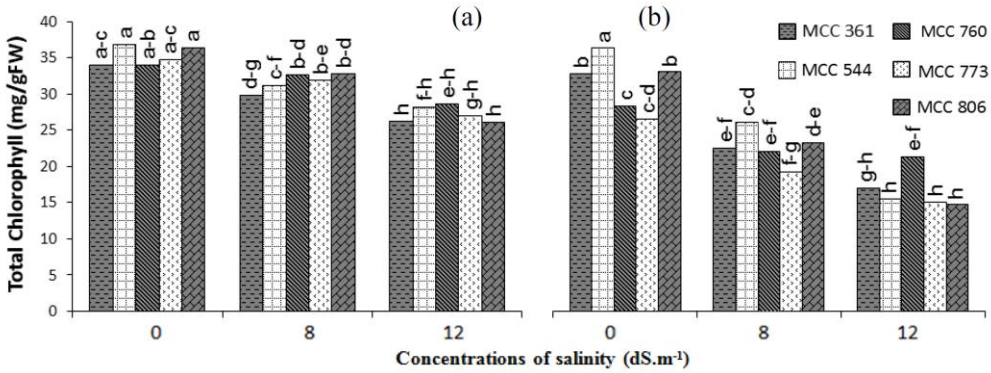

Figure 6. Changes of total Chlorophyll content under three salinity concentrations (0, 8 and 12 dS.m^{-1}), in 21-day old seedlings (a) and 28-day old seedlings (b). Means in columns with at least one letter in common in the range are not significantly different ($p \leq 0.05$).

The status of total chlorophyll content were measured to give an insight into photosynthetic capabilities. In consistent with other studies (Hernandez *et al.*, 1995), a higher chlorophyll degradation was observed in salt-sensitive chickpea genotypes. The rate of decline and loss of total chlorophyll contents increased with higher NaCl concentrations and it was found to be significantly up to 24% less in MCC760, in 28 DAS seedlings (Fig. 6 and 7). 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 as observed in this study (Fig. 7) (Yadav *et al.*, 2011). Observed degradation may be due to increasing of destructive enzymes called chlorophyllase (Rahdari *et al.* 2012). Pigments system reduction is attributed to weakening the protein-pigment-lipid complex induction or elevated chlorophyllase enzyme activity (Turan *et al.*, 2007). The reduction of total chlorophyll amounts in chickpea upon salt stress was also reported (Beltagi, 2008 ؛ Mudgal *et al.*, 2009).

Correlation between biochemical parameters

To study mechanism of tolerance, after initial screening of genotypes, correlations of biochemical parameters was compared between tolerant and susceptible 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 result in membrane stability (0.26* and 0.61** correlations values), meanwhile membrane damage in susceptible genotypes could be due to decrease of necessary proteins and carbohydrates since the correlation values were negative.

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.

	Proline	*MDA*	*Protein*	*Carbo*	*MSI*	*Chl*	*Ro/Sh*
Proline	1	**0.63 ****	**0.60****	**0.80****	**- 0.21***	**- 0.58****	**0.33***
MDA	0.97**	1	**0.26***	**0.57****	**- 0.46****	**- 0.81****	**0.51****
Protein	0.01	0.001	1	**0.61****	**0.26***	**- 0.46****	**0.02**
Carbo	0.07	0.13	0.001	1	**- 0.25***	**- 0.52****	**0.23***
MSI	- 0.29*	- 0.34*	- 0.14	- 0.09	1	**0.52****	**0.01**
Chl	- 0.78**	- 0.85**	0.001	- 0.29*	0.40**	1	**- 0.37****
Ro/Sh	- 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.

Chlorophyll degradation and MDA accumulation in leaves of tolerant genotypes could 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 might be due to less photosynthesis rate (Kafi *et al.*, 2011).

SDS-PAGE analysis

Comparative evaluation of changes in protein profile was performed based on previous findings using SDS-PAGE analysis (Fig. 7). Clear differences pattern in protein changes was seen between tolerant and susceptible genotypes on the polyacrylamide gels from presence or absence bands to varied intensity of expression. A 45 kDa protein band in MCC544 showed 3, 1.8 and 0.8 fold more intensity than MCC806 at 48, 96 and 168 h respectively. This suggests that the earlier expression of this category of proteins may have a role in tolerance response. Absence or presence of some bands may also indicate a functional involvement in stress response (Fig 7). The results showed that protein expression in tolerant genotype started to decrease after 98 h but in susceptible one increased after this time point. This could be considered as a key point in protein pattern changes either in tolerant or susceptible genotypes. It seems that tolerance reaction might be due to more rapid synthesis or less degradation of responsive proteins to salinity especially for higher molecular weight proteins.

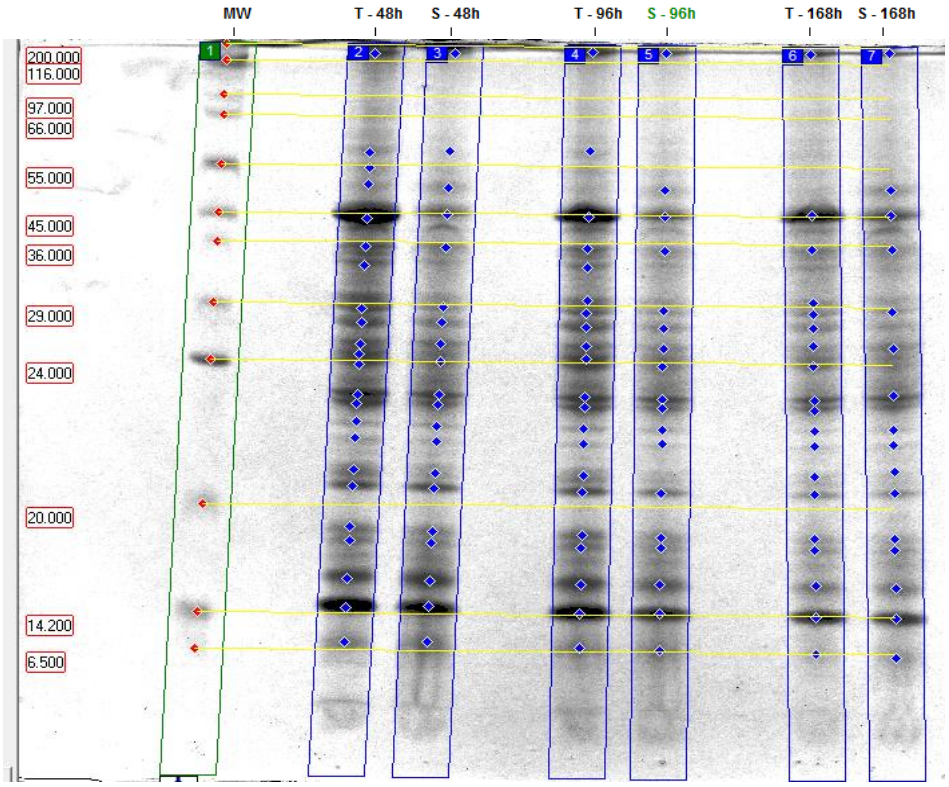

Figure 7. SDS-PAGE of tolerant (T; MCC544) and susceptible (S; MCC806) chickpea genotypes during different salinity periods (48, 96 and 168 h) along with standard MW.

The relationship of protein profile in each genotype and time point is illustrated in clustering analysis in figure 8. Tolerant genotype had a less changes over time especially in initial time point, confirming our previous findings. 96 and 168 hours salinity periods resulted in more protein changes compared to 48 and 96 in susceptible one. Overall based on this cluster analysis, salinity had the most impact on protein accumulation after 96 hour and responses of seedlings before this time point might be important in tolerance mechanism (Wang *et al.*, 2009).

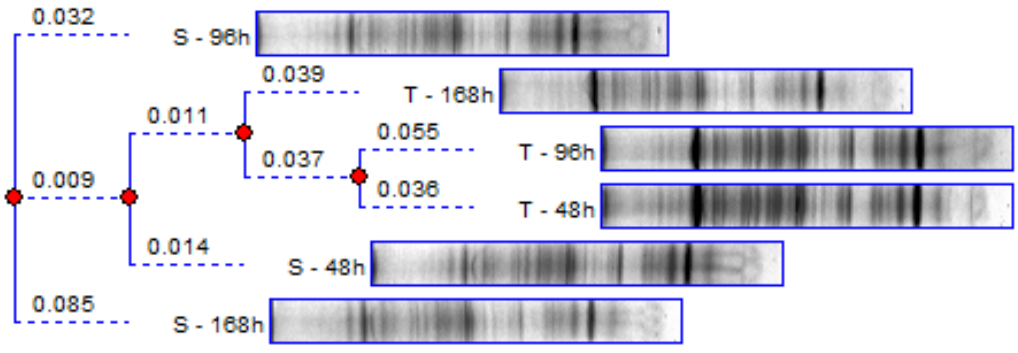

Figure 8. Cluster analysis of proteins in chickpea genotypes (T: MCC544 or S: MCC806) under salinity periods (48, 96 and 168 h).

CONCLUSIONS

Generally, tolerance in saline condition might be due to more rapid accumulation of proteins with higher molecular weight and also more organized and coordinated pattern changes in biochemical parameters. Furthermore, according to susceptible genotype response in this study, irregular changes in protein profile or inability to rapid accumulation of responsible proteins may be possible cause for susceptibility in saline condition (Table 1 and Fig. 8).

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