Biochemical performance and protein profile of sensitive and tolerant varieties of chickpea under salinity

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ABSTRACT
Chickpea varieties (BG-256 and CSG-8962) were subjected to salt stress (50mM, 100mM and 150mM of NaCl) in a pot experiment. Untreated plants served as control. Plants were analyzed from 20 DAS up to 60 DAS at ten-day interval. Salt stress significantly reduced growth parameters like biomass, net assimilation rate, relative water content and biochemical parameters viz., total nitrogen and protein content of both the varieties. However, decrement was more pronounced in sensitive (BG-256) than in tolerant (CSG-8962) variety. Proline content increased with increase in salt exposure in both the varieties. SDS-PAGE of the protein reveal large amount of protein degradation in plants treated with high concentration of salt.

Key words: BG-256, Biochemical parameters, CSG-8962, Protein profiling, Salinity.

INTRODUCTION
Chickpea (Cicer arietinum L.) is an important pulse crop (family Fabaceae, sub-family Papilionoideae), grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and proteins (20%) and protein quality is considered to be better than other pulses (Jukanti et al., 2012). It plays an important role in maintenance of the soil fertility through nitrogen fixation (Varshney et al., 2009). Besides, the use of legumes as green manure can potentially increase crop productivity in saline environments and thus contribute to the sustainability of agricultural systems. (Bruning et al., 2015).

A variety of abiotic stresses significantly affects growth and productivity of plants. Of various abiotic stresses, soil salinity is known to cause considerable crop losses (Ashraf et al., 2008). India is the largest producer, consumer and processor of the pulses in the world (Srivastava et al., 2010). Among the pulses chickpea is the most important crop representing about 27% of the land area under pulse, which contributes 33% of the total pulse production in India (Anonymous, 2000).

Salinity has significant effect on net assimilation rate (Praveen et al., 1990). The increase in salt stress causes a reduction in water uptake due to excess amount of salt present in the soil water solution due to decrease in water potential and water content (Wang et al., 2012). Amrinejad et al. (2017) reported that the proline content increases in pepper plants treated with salt concentrations. Protein content can also be affected negatively or positively, by salt stress. The results of certain studies (Chen et al., 2007; Kapoor and Srivastava, 2010) report a decrease, or increase, in protein content in Vigna plants treated with different salt concentrations. Salt stress causes an induction and inhibition of some polypeptides in the leaves and the changes in protein synthesis can be determined as new synthesis, complete loss, increase or decrease. Plants under high salt concentration synthesizes osmolytes and few specific proteins called stress responsive proteins in order to cope with stress (Srivastava and Shahi, 2017).

It is estimated that, worldwide, 800 mha of land and 32 mha of agricultural land are salt-affected (FAO, 2015). In order to enhance the productivity of crop plants, improving their salt tolerance is an effective measure to make marginal areas agriculturally productive (Das and Kundagrami, 2018; Karan and Subudhi, 2012). Hence, it is important to understand the physiological, biochemical and molecular mechanisms evolved by plants to cope with salt stress (Gharsallah et al., 2016).

Chickpea is considered a salt sensitive species, but some genetic variation for salinity tolerance exists. The present investigation was therefore conducted to explore the effect of salt stress on morphophysiological aspects of two varieties of chickpea, viz. BG-256 (salt sensitive) and CSG-8962 (salt tolerant). Electrophoretic analysis of leaf protein of both the species were also performed to reveal the protein profiles.

MATERIALS AND METHODS
Healthy seeds of chickpea were surface sterilized with ethanol for 5 min followed by thorough wash with distilled water. Surface sterilized seeds were then inoculated
with 96 hour grown culture of *Mesorhizobium ciceri*. The inoculated seeds were sown in earthenware pots containing sterilized sand. These pots were irrigated with saline water containing 50 mM, 100 mM and 150 mM NaCl. The untreated plants served as control. Plants were supplied with Hoagland’s nutrient solution weekly. All physiological and biochemical parameters were recorded from 20 DAS (days after stress) to 60 DAS at 10-day interval for each treatment. Randomized complete block design (RCBD) was used with 3 replications.

Oven dried plants (65 ± 2°C) were used for estimation of biomass. The Net Assimilation Rate (NAR) was calculated as described by Watson (1958) by the following formula:

$\text{NAR} = \frac{(W_2 - W_1) (log A_2 - log A_1)}{(t_2 - t_1) (A_2 - A_1)}$

Where, $A_1$ = leaf area/plant at $t_1$ stage; $A_2$ = leaf area/plant at $t_2$ stage; $W_1$ = Biomass at $t_1$ stage; $W_2$ = Biomass at $t_2$ stage

Relative water content (RWC) was calculated as described by Schonfeld et al. (1988) by the following formula –

$\text{RWC}% = \frac{\text{fresh weight (fw)} - \text{dry weight (dw)}}{\text{turgid weight (tw)} - \text{dry weight (dw)}} \times 100$

Proline was estimated by the method of Bates et al. (1973). Samples were homogenized in 10 mL 3% (w/v) sulfosalicylic acid, and proline was assayed by the acid ninhydrin method. The absorbance was measured spectrophotometrically at 520 nm.

Total nitrogen content was estimated by the method of Doneen (1932). Powdered samples were kept in boiling test tube. To each fraction, (insoluble and total) 1.0 ml concentrated sulphuric acid (containing 5% salicylic acid, w/v) was added. It was then heated gently until fumes appeared. A small pinch of sodium thiosulfate (Na₂S₂O₃) was added to it. The tube was cooled to room temperature and then 1.0 ml of perchloric acid (containing 0.1% CuSO₄.5H₂O w/v) was added. The tubes were again heated for a period till the content become clear. Each digested sample was cooled and diluted to 100 ml with distilled water. To 1.0 ml of this solution 1.0 ml Nessler’s reagent was added. The absorbance of pale yellow colour so developed was measured at 440 nm.

For measurement of protein content in dried leaves the amount of insoluble nitrogen fraction was multiplied by a factor of 6.25.

Protein profiling was done at 20, 40 and 60 DAS from the leaf samples obtained from both sensitive and tolerant varieties of chickpea plants at different NaCl concentration.

**Sample preparation from chickpea leaves for SDS Polyacrylamide gel electrophoresis:** Leaf protein samples were prepared by grinding 1g of fresh leaves in 1ml 0.1M Tris buffer (pH 7.5) containing 50 mg polyvinyl pyrrolidine. These were then centrifugated at 10,000g at 4°C for 15 min. The supernatant was used for sodium dodecyl sulphate polyacrylamide gel Electrophoresis (SDS-PAGE).

**Protein profiling using SDS-PAGE:** Protein profiling of samples was performed as described by Laemmli (1970). Electrophoresis was performed on 12% SDS-PAGE (5% stacking and 12% resolving gel) using vertical gel apparatus. The protein extract was transferred to an equal volume of gel loading dye, heated at 100°C for 3 min, cooled and used for SDS-PAGE. 30 µL of sample was loaded in a separate well and 5µL of molecular marker was loaded in a separate well. Electrophoresis was performed at 75V for first two hours and then 90V for the third hour. At the end of electrophoresis gels were stained with Coomassie Blue G-250 dye and then destained overnight. Finally, gels were photographed using gel documentation system.

The data have been statistically analyzed. Least significant difference (LSD) has been calculated for data where F test was found significant using Graph Pad Prism software.

**RESULTS AND DISCUSSION**

Biomass of plants increased from 20 DAS upto 60DAS and decrease with increasing salt concentration. The decrease was more in sensitive than in tolerant variety with maximum reduction at 150 mM NaCl treatment (Fig 1a and 1b). Singh *et al.* (2017) working on *Sesame indicum* reported that plant grown in non-saline soils had higher dry matter as compared to plants grown in saline soil. The results were agreement with findings of Shahi and Srivastava (2016) in mungbean. Pegu *et al.* (2018) working on green gram cultivars reported more reduction in plant dry mass in salt sensitive cultivars as compared to tolerant ones under NaCl stress.

NAR increased from 20 DAS upto 60 DAS in both the varieties of chickpea under all treatments. There was a reduction in NAR of plants with increasing salt concentration in both varieties, reduction being more pronounced in sensitive than tolerant one. Maximum decrement was observed at 150mM NaCl treatment (Table 1a and 1b). The observed reduction in NAR may be due to combination of slower growth and development as a result of osmotic stress and inhibition of photosynthetic apparatus (Srivastava and Shahi, 2018). Another reason might be restricted availability of essential nutrients and decreased photosynthetic efficiency (Datta, 1994).

RWC increased from 20 DAS upto 40 DAS followed by a gradual decline. RWC decreased significantly under salt stress and maximum decrement was observed at 150mM NaCl treatment in both varieties. More decrement was observed in sensitive than in tolerant variety (Fig 2a and 2b). Similar results were obtained by Ramana *et al.* (2012) in four soyabean cultivars in which reduction was
Table 1a: Net assimilation rate (mg cm\(^{-2}\)/10 day interval) at different age of growth in BG-256 variety of chickpea under different NaCl concentrations.

<table>
<thead>
<tr>
<th>Treatments (NaCl)</th>
<th>Plant Age (DAS)</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-30</td>
<td>30-40</td>
<td>40-50</td>
<td>50-60</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.021±0.003*</td>
<td>0.016±0.001*</td>
<td>0.013±0.002*</td>
<td>0.007±0.001*</td>
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<tr>
<td>50mM</td>
<td>0.024±0.004</td>
<td>0.02±0.003</td>
<td>0.017±0.002</td>
<td>0.011±0.004</td>
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<tr>
<td>100mM</td>
<td>0.027±0.003</td>
<td>0.023±0.002</td>
<td>0.019±0.002</td>
<td>0.016±0.004</td>
<td></td>
</tr>
<tr>
<td>150mM</td>
<td>0.028±0.005</td>
<td>0.024±0.003</td>
<td>0.021±0.002</td>
<td>0.015±0.001</td>
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</tbody>
</table>

*Mean ± Standard Deviation (n=3)

Table 1b: Net assimilation rate (mg cm\(^{-2}\)/10 day interval) at different age of growth in CSG-8962 variety of chickpea under different NaCl concentrations.

<table>
<thead>
<tr>
<th>Treatments (NaCl)</th>
<th>Plant Age (DAS)</th>
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<tbody>
<tr>
<td></td>
<td>20-30</td>
<td>30-40</td>
<td>40-50</td>
<td>50-60</td>
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</tr>
<tr>
<td>Control</td>
<td>0.024±0.002*</td>
<td>0.018±0.001*</td>
<td>0.015±0.001*</td>
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<tr>
<td>50mM</td>
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<td>0.024±0.003</td>
<td>0.020±0.003</td>
<td>0.014±0.003</td>
<td></td>
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<tr>
<td>100mM</td>
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<td>0.029±0.003</td>
<td>0.025±0.002</td>
<td>0.019±0.003</td>
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<tr>
<td>150mM</td>
<td>0.036±0.002</td>
<td>0.030±0.004</td>
<td>0.027±0.002</td>
<td>0.018±0.005</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± Standard Deviation (n=3)

more articulate in sensitive cultivar than the tolerant one. This work was also in consideration with the observation of Rahneshan et al. (2018) in pistachio rootstocks and Garg and Singla (2016) in chickpea cultivars.

In the present investigation, Proline content increased with increasing salt concentration and increment was more in tolerant than in sensitive variety (Fig 3a and 3b). Maximum proline was observed at 40 DAS in 150mM NaCl treated plants of tolerant variety. Our result is in consideration with those obtained by Sivasankaramoorthy (2013) in chickpea and Hasan et al. (2015) in wheat. Sehrawat et al. (2013) reported that accumulation of proline increased significantly with increase in salt stress in mungbean plants but increase was more pronounced in tolerant than in sensitive cultivar.

The results indicated reduction in total nitrogen content with increase in salt concentration, reduction being more pronounced in sensitive than in tolerant variety. Total nitrogen content increased from 20 DAS upto 40 DAS followed by a gradual decline up to 60 DAS (Fig 4a and 4b). Perez-Alfocea et al. (2015) reported that the total nitrogen content decreases with increasing salt concentrations in salt sensitive genotypes of tomato but no significant decrease were observed in salt tolerant variety. This work is in agreement with the observations of Bybordi (2010) in canola cultivars.

Fig 5a and 5b shows the effect of salinity in BG-256 and CSG-8962 varieties of chickpea respectively. Protein content increased from 20 DAS upto 40 DAS followed by a gradual decline up to 60 DAS. The results indicated reduction...
**Fig 2a:** *Cicer arietinum*: Relative Water Content of plants at different age of growth in BG-256 variety of chickpea under different NaCl concentrations.

**Fig 2b:** *Cicer arietinum*: Relative Water Content of plants at different age of growth in CSG-8962 variety of chickpea under different NaCl concentrations.

**Fig 3a:** *Cicer arietinum*: Proline Content of plants at different age of growth in BG-256 variety of chickpea under different NaCl concentrations.

**Fig 3b:** *Cicer arietinum*: Proline Content of plants at different age of growth in CSG-8962 variety of chickpea under different NaCl concentrations.

**Fig 4a:** *Cicer arietinum*: Total Nitrogen Content of plants at different age of growth in BG-256 variety of chickpea under different NaCl concentrations.

**Fig 4b:** *Cicer arietinum*: Total Nitrogen Content of plants at different age of growth in CSG-8962 variety of chickpea under different NaCl concentrations.
in protein content with increase in salinity and the reduction being more prominent in sensitive than in tolerant variety. Kumar et al. (2010) reported that the total protein content of salt tolerant genotypes of Oat increases with increasing salt concentrations but at very high concentrations of salt protein content decreases. However, in salt sensitive genotypes a slight increment in protein content were observed at very low concentration of salt exposure but it reduces upon increasing salinity.

Initially at 20 DAS, all the samples showed similar band pattern for protein profiling. There was no noticeable difference in band pattern obtained from SDS-PAGE from all the samples of both varieties under all treatments (Plate 1). At 40 DAS the result of protein profiling showed a band (molecular weight ranging between 95 and 72 kDa) in the control plants of both the varieties which was absent in the plants given NaCl stress (Plate 2). The same protein band was also synthesized in the plants treated with 50mM NaCl at 60 DAS along with control, but was absent in plants treated with 100mM and 150mM NaCl solution. The protein profiling of the samples done at 60 DAS showed large amount of protein degradation in the plants treated with high salt concentration (100mM and 150mM) in both varieties of chickpea. Whereas the control plants as well as treated with 50mM NaCl showed normal band pattern with very little protein degradation. Maximum protein degradation was observed in sensitive variety given highest salt stress i.e., 150mM. (Plate 3). Depressed protein synthesis and acceleration in its degradation in plants in response to salt stress has been reported by Elavumottil et al. (2003). Our result is in accordance with the results Badran et al. (2015) in alfalfa. Accumulation and/or less degradation of proteins of high molecular weights was also observed in chickpea through SDS-PAGE analysis (Arefian et al., 2014). Appearance of two additional protein bands may be the biochemical moderator developed in plants under salt stress condition. Disappearance of the protein bands may be interpreted as the ‘turning off’ of protein synthetic genetic machinery in response to salt. It is more likely, however, that the ‘disappeared’ proteins in response to stress are a result of their denaturation (Meratan et al., 2008).

**Fig 5a: Cicer arietinum:** Total Protein Content of plants at different age of growth in BG-256 variety of chickpea under different NaCl concentrations.

**Fig 5b: Cicer arietinum:** Total Protein Content of plants at different age of growth in CSG-8962 variety of chickpea under different NaCl concentrations.

**Plate 1:** SDS-PAGE resolved band pattern of leaf proteins of both varieties of chickpea at 20 DAS of plant growth under different salt concentrations.
CONCLUSION

High salinity adversities can be seen on physiological and biochemical behavior of plants. When the plants are exposed to mild stress, physiological and biochemical pattern of the plant changes in order to overcome the adverse effects. The tolerant variety shows better adaptability towards salinity. Salinity resulted in formation of new bands and disappearance of others in the protein profiling of chickpea plants. Such an understanding will be helpful in future breeding program for developing salt tolerant chickpea genotypes.

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