Study the effect of salt stress on physio-biochemical traits of wheat

Basant Kumar Dadrwal, DL Bagdi, BL Kakralya, Surendra Kumar Choudhary and Pinki Dadrwal

Abstract
A pot experiment was conducted during rabi season, 2018 in the cage house at Department of Plant Physiology, S.K.N. College of Agriculture, Jobner, Rajasthan to study the effect of salt stress on physio-biochemical traits of wheat. Wheat cultivars namely Raj-4037 (salinity susceptible) and Raj-3077 (Salinity tolerant) were grown in ceramic pots under salinity conditions (0, 4 and 8 dSm⁻¹). Control plants were provided normal water. Different physio-biochemical observations were recorded at 55 and 75 days after sowing in pot conditions. Result revealed a significant decrease were recorded in chlorophyll content, protein content, relative water content, cell membrane stability whereas, proline content and reducing sugar increased with salt stress up to EC 8 dSm⁻¹ in both the cultivars at 55 and 75 DAS. Reduction in physio-biochemical traits contributing parameters on account of salt stress was more in cultivar Raj-4037 whereas, proline content and reducing sugar increased. On the basis of research findings genotype Raj-3077 observed most salt tolerant and the tolerance was mediated by physio-biochemical traits characteristics.

Keywords: Salinity, proline, chlorophyll content, wheat, Salinity tolerant, salinity susceptible

Introduction
Wheat (Triticum aestivum L.) is one of the most important staple food crop of the world as well as India. It is believed to be native of South Western Asia. It is an annual herb belonging to the family Gramineae. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land has been damaged by salt in the worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Munns and Tester, 2008) [14]. Salinity reduces the ability of plants to take up water, causing a reduction in growth along with a suite of metabolic changes. A metabolic response to salt stress is the synthesis of compatible osmolytes. These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity. Salt stress affects the various physiological processes of plants which reduces the yield and quality of produce. Therefore, osmotic adjustment has proved more important salt tolerance mechanism in wheat, where plants are forced to decrease their internal water potential via, accumulating ions in the vacuoles. Proline, sugar, glycine betaine etc. are some of the good osmoprotectants accumulate in the cytoplasm. The process allow turgor driven process such as stomatal opening at reduced rate by maintaining the water balance of the plant cells (Blum, 1989 and Gupta et al., 2001) [6, 10]

Negrao et al. (2017) [17] observed that the soil salinity is a major abiotic constraint affecting crop yield such as relative growth rate, water relations, transpiration, transpiration use efficiency, ionic relations, photosynthesis, senescence, yield and yield components. Salinity tolerance is complex and involves many genes, but progress has been made in studying the mechanisms underlying a plant’s response to salinity. Alam et al. (2015) [2] reported that the salinity effect was evaluated on the basis of biomass yield reduction, physiological attributes. Aggravated salinity stress caused significant reduction in all measured parameters and the highest salinity showed more detrimental effect compared to control as well as lower salinity levels. Overall, salinity stressed among all purslane accessions considering biomass production, physiological growth and anatomical development Ac9 (salt-tolerant) was the best purslane accession and Ac13 (salt-susceptible) was the most affected accession. Ghosh et al. (2016) [9] studies that the Salinity has been a key abiotic constraint devastating crop production worldwide. The differential responses of rice, in salt toxicity enumerating the detailed
morphological, physiological, biochemical and molecular changes. Rice is susceptible to salinity specifically at the early vegetative and later reproductive stages and the response of the crop to excessive salt toxicity at biochemical and molecular level as well as physiological level. Attempts in understanding salt tolerance mechanisms has revealed several key enzymes and altered biochemical pathways inferring resistance to crop plants against salt stress. Kumar (2017) [12] reported that the study aimed to investigate physio-biochemical, molecular indices and defense responses of selected wheat cultivars. Better antioxidant potential, membrane stability, increased accumulation of osmolytes/phytocompounds, and higher K+/Na+ ratio under NaCl stress identified Kharchia-65 to be the most salt-tolerant cultivar. By contrast, increased MDA level, reduced soluble sugar, proline, total chlorophyll, total phenolics contents and lower antioxidant potential in HD-2329 to be sensitive to the stress. Orabi et al. (2013) [18] revealed that salt treatments provoked oxidative stress in faba bean (Vicia faba L.) Plants as shown by the increase in lipid peroxidation and electrolyte leakage and consequently negatively affected growth and yield criteria. Jamil and Rha (2013) [11] reported that the net CO2 assimilation, stomatal conductance, transpiration rate and intrinsic water-use efficiency decreased remarkably with increasing NaCl concentration in mustard cultivar while water use efficiency increased at 50 mM NaCl but then reduced. Chlorophyll content enhanced considerably with the increasing NaCl concentration. There was an increase in the concentration of total protein content with the corresponding increase in NaCl level up to 100 mM.

2. Materials and Methods
Investigate the effect of salt stress on yield and yield attributes of wheat cultivars namely Raj-3077 salinity tolerant and Raj-4037 salinity susceptible will be screened out in pot conditions. Seeds were raised in seventy two cemented pots filled with about 15 Kg of wellmixed FYM soil in each pot. The crop will be irrigated with saline irrigation water one liter to each pot of EC 0 (Tap water), 4 and 8 dS m-1 prepared by mixing of NaSO4, NaCl, CaCl2, and MgCl2 salts in 3:1 ratio of chloride and sulphate up to maturity. Chlorophyll “a” and “b” content as mg g-1 fresh weight were estimated according to the method of Arnon (1949). Sample extract was prepared from 100 mg of leaf samples in 5 ml of 85% acetone and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25 ml measuring cylinder. The residue was re-extracted with 5 ml of acetone, centrifuged and the supernatant was transferred to the measuring cylinder. Final volume of the supernatant was made to 10 ml with 85% acetone. Finally, the optical density (OD) of chlorophyll ‘a’ and ‘b’ was measured at 663 nm and 645 nm. The total chlorophyll content was calculated by the formula:

Chlorophyll ‘a’ (mg g-1) = (12.7 A663) - 2.69 (A645) x \frac{V}{1000 \times \text{leaf wt. (g)}}

Chlorophyll ‘b’ (mg g-1) = (22.9 (A663) - 4.68 (A645) x \frac{V}{1000 \times \text{leaf wt. (g)}}

The relative water content (RWC) was calculated by the formula as:

R.W.C. (%) = \frac{\text{Fresh weight – Dry weight}}{\text{Turgid weight – Dry weight}} \times 100

Membrane stability (%) was calculated by taking the electrical conductivity of leaf leachates in double distilled water at 40 and 100° C by following the method of Sairam (1994). Mature leaf were cut into small pieces and then taken (0.5 g) in test tubes having 10 ml of double distilled water in two sets. One set was kept at 40° C for 30 min and another set at 100° C in boiling water bath for 15 min and their respective electric conductivity’s C1 and C2 were measured by conductivity meter (Adaw-A260, Germany). Membrane stability (%) = 1-C1/C2 x 100

Where,
C1 = Electric conductivity’s at 40° C
C2 = Electric conductivity’s at 100° C

Free proline (mg g-1 fresh weight of leaf) was determined using the method of Bates et al. (1973) Protein (mg g-1 fresh weight of leaf) was measured by method of (Lowry et al., 1951). Reducing sugar was estimated in the ethanol soluble fraction as described by Nelson, (1994). 0.5 ml aliquot was taken intact tube and volume was made to 1 ml with double distilled water. After adding 1 ml copper reagent, the tube were bath be 20 minutes and then to cooled at room temperature. Added 1 ml aresnomolybdate reagent then to each test tube. Final volume was made to 10 ml with double distilled water and the absorbance was measured at 570 nm. Protein (mg g-1 fresh weight of leaf) was measured by method of (Lowry et al., 1951). 100 mg plant leaves were homogenized in 5 ml phosphate buffer (0.1 N pH 7.5). The supernatant was collected after centrifugation at 5000 rpm for 10 min and final volume was made to 5 ml with buffer. Aliquot (0.2 ml) was taken in test tube at the same time in a series of test tubes 0.2, 0.4, 0.6, 0.8 and 1.0 ml of protein solution were prepared by dissolving 10 mg of bovin serum albumin (BSA) in 100 ml saline solution. In each test tube the volume was made to 1.0 ml with dH2O. A tube with 1.0 ml dH2O served as control. After adding 5.0 ml of alkaline copper solution in each test tube, the mixture was kept at room temperature for 10 min. This was followed by addition of 0.5 ml of diluted folin ciocalteau’s reagent. The mixture was incubated at room temperature for 30 min under dark and absorbance of the blue colour was recorded at 660 nm using spectrophotometer. The quantity of protein in the 100 mg leaf sample was then calculated using the standard curve. The reagents were made as follows: (A) Sodium carbonate- (2% in 0.1 N sodium hydroxide) (B) Copper sulphate- (0.5% in 1% Na-K tarterate) (C) Alkaline copper solution was prepared by mixing solution A and B in the ratio of 50 : 1 at the time of use. (D) The commercial Folin-ciocalteau reagent was diluted with equal volume of water. Reducing sugar was estimated in the ethanol soluble fraction as described by Nelson, (1994). 0.5 ml aliquot was taken intact tube and volume was made to 1 ml with double distilled water. After adding 1 ml copper reagent, the tube were bath be 20 minutes and then to cooled at room temperature. Added 1 ml aresnomolybdate reagent then to each test tube. Final volume was made to 10 ml with double distilled water and the absorbance was measured at 570 nm. All the data were statistically analysed using Completely Randomized Design (CRD) with three replications.
3. Results and discussion

Table 1: Effect of salinity on chlorophyll content, relative water content and cell membrane stability of wheat

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll (mg/g f.w.)</th>
<th>Relative water content (%)</th>
<th>Cell membrane stability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>55 DAS</td>
<td>75 DAS</td>
<td>55 DAS</td>
</tr>
<tr>
<td>Raj-3077</td>
<td>2.23</td>
<td>2.72</td>
<td>71.21</td>
</tr>
<tr>
<td>Raj-4037</td>
<td>1.95</td>
<td>2.43</td>
<td>66.56</td>
</tr>
<tr>
<td>S.Em.+</td>
<td>0.03</td>
<td>0.04</td>
<td>1.11</td>
</tr>
<tr>
<td>C.D.(P=0.05)</td>
<td>0.07</td>
<td>0.09</td>
<td>3.30</td>
</tr>
</tbody>
</table>

 Chlorophyll content (mg g⁻¹ fresh weight of leaf) : Varietal response; It is evident from the data in Table 1 revealed that the increase in chlorophyll content of Raj-3077 was found significantly more than Raj-4037 under non stress and salt stress conditions. The per cent increase in chlorophyll content of Raj-3077 was recorded 14.35 and 11.93 than Raj-4037 at 55 and 75 DAS, respectively. Further examination of data presented in the above table revealed that salt stress caused significant reduction in chlorophyll content up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. The decrease in chlorophyll content at EC 4.0 and EC 8.0 dSm⁻¹ was recorded 9.05, 24.56 and 14.02, 21.94 per cent over control at both the stages, respectively. Relative water Content (%): Varietal response; The data in Table 1 revealed that the RWC was recorded significantly higher in Raj-3077 than Raj-4037 at 55 and 75 DAS under non stress and salt stress conditions. The increase in RWC of Raj-3077 was 6.98 and 7.45 per cent than Raj-4037 at both the stages. Effect of salinity; Data further indicated that RWC decrease significantly with increasing level of salinity up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. The decrease in RWC at EC 4.0 and EC 8.0 dSm⁻¹ was recorded 1.85, 4.37 and 2.31, 4.65 per cent over control at both the stages, respectively. Cell membrane stability (CMS %): Varietal response; The data in Table 1 revealed that the cell membrane stability was recorded significantly higher in Raj-3077 than Raj-4037 at 55 and 75 DAS under non stress and salt stress conditions. The increase in cell membrane stability of Raj-3077 was 6.06 and 4.67 per cent than Raj-4037 at both the stages. Effect of salinity; Data further indicated that cell membrane stability decrease significantly with increasing level of salinity up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. The decrease in cell membrane stability at EC 4.0 and EC 8.0 dSm⁻¹ was recorded 5.63, 19.19 and 4.62, 15.42 per cent over control at both the stages, respectively.

Table 2: Effect of salinity on Proline, Protein and reducing sugar of wheat

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Proline (µg/g f.w.)</th>
<th>Protein (mg/g f.w.)</th>
<th>Reducing sugar (mg/g f.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>55 DAS</td>
<td>75 DAS</td>
<td>55 DAS</td>
</tr>
<tr>
<td>Raj-3077</td>
<td>36</td>
<td>57</td>
<td>19.91</td>
</tr>
<tr>
<td>Raj-4037</td>
<td>28</td>
<td>50</td>
<td>18.35</td>
</tr>
<tr>
<td>S.Em.+</td>
<td>0.09</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td>C.D.(P=0.05)</td>
<td>0.22</td>
<td>0.31</td>
<td>0.76</td>
</tr>
</tbody>
</table>

 Proline content (µg g⁻¹ fresh weight of leaf): Varietal response; A perusal of data in Table 2 revealed that the increase in proline content of Raj-3077 was found significantly more than Raj-4037 under both non stress and salt stress conditions. The per cent increase in proline content of Raj-3077 was recorded 28.57 and 14.00 over Raj-4037 at 55 and 75 DAS, respectively. Further examination of data presented in the above table further revealed that salt stress significantly increased proline content in leaves up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. The increase in proline content at EC 4.0 dSm⁻¹ was 60 and 130 per cent and at EC 8.0 dSm⁻¹, it was registered 25.58 and 65.11 per cent over control at both the stages of investigation, respectively. Protein content (mg g⁻¹ fresh weight of leaf); Varietal response; Data from Table 2 revealed that the cultivar Raj-3077 registered significantly higher protein content over Raj-4037 under both non stress and salt stress conditions. The per cent increase in protein content of Raj-3077 was 8.50 and 6.90 than Raj-4037 at 55 and 75 DAS. Effect of salinity; Further examination of data given in above table revealed that a significant decrease in protein content in plant leaves was recorded due to salinity up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. At EC 4.0 and EC 8.0 dSm⁻¹ decreased in protein content in leaves to the extent of 6.69, 16.75 and 1.03, 15.78 per cent over control was recorded at both the stages of investigation, respectively. Reducing Sugar (mg/g f.w.): Varietal response; Data from Table 2 revealed that the cultivar Raj-4037 registered significantly higher reducing sugar content over Raj-3077 under both non stress and salt stress conditions. The per cent increase in reducing sugar content of Raj-4037 was 12.11 and 13.74 than Raj-3077 at 55 and 75 DAS. Effect of salinity; Further examination of data given in above table revealed that a significant increase in reducing sugar content in plant leaves was recorded due to salinity up to EC 8.0 dSm⁻¹ at 55 and 75 DAS. At EC 4.0 and EC 8.0 dSm⁻¹ increase in reducing sugar in leaves to the extent of 14.94, 19.47 and 10.58, 14.37 per...
A significant decrease in chlorophyll content was observed under salt stress conditions at 55 and 75 days after sowing. The Raj-3077, a salt tolerant genotype, showed lesser reduction in chlorophyll content than Raj-4037 (Table 1). Higher chlorophyll content of Raj-3077 reflects its relative tolerance to salt stress. In current study, the chlorophyll contents significantly decreased under elevated salt stress, as the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in several plants, such as pea (Ahmad and Jhon, 2005) [1], wheat (Ashraf et al., 2002) [4] and rice (Anuradha and Rao, 2003) [3]. Sairam et al. (2002) [19] revealed that salinity induced decrease in chlorophyll and carotenoids were significantly higher in KRL 19 than more tolerant Kharchia 65. Similarly, Sairam et al. (2002) [20] reported that reduction of chlorophyll content in a tolerant wheat cultivar was lower than in a sensitive one. Membrane stability decreased under salinity stress in both the genotypes at both the stages of sampling (Table 1). Higher membrane stability was retained by tolerant cultivar Raj-3077 compared to susceptible Raj-4037 under salt stress and non stress conditions at both stages of investigation. The results are in accordance with the findings of Sairam and Srivastava (2002) [20] they found that salinity caused to decrease membrane stability index in two wheat genotypes but the reduction was more pronounced in susceptible one (Raj-4037) than tolerant (K-65) genotype. It has also been reported that salinity stress decreased significantly membrane stability index of wheat (Sairam et al. 2002) [19]. Soil salinity significantly reduced protein content in the grain while proline accumulation was stimulated in the leaves. The increase in leaf proline was relatively greater in the tolerant genotypes than the susceptible ones (Sharma et al. 2003) [21]. Our studies also noticed that salt stress significantly increased the proline content of both wheat genotypes at 55 and 75 days after sowing. The results showed that the increase in proline content was higher in salt tolerant cultivar Raj-3077 than in comparison to salt susceptible cultivar Raj-4037 at both the stages of investigation. Free proline is known to accumulate in response to biotic and abiotic stresses and has been shown to protect plants against free radical induced damage as reported by Matysik et al. (2002) [13]. This is because proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive cultivars of many crops (Misra and Gupta, 2005) [16]. A significant decrease in protein content was observed under salt stress conditions at 55 and 75 days after sowing in both wheat genotypes. The Raj-3077 salt tolerant genotype, showed lesser reduction in protein content than Raj-4037 at both the stages of study. Nuclic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparison to control (Bera et al. 2006) [5].

4. Conclusion
Salt stress registered a significant decrease in were recorded in chlorophyll content, protein content, relative water content, cell membrane stability whereas, proline content and reducing sugar increased. Cultivar Raj-3077 perform better over Raj-4037 under non stress and salt stress conditions. Further, the results concluded that cultivar Raj3077 (Salinity tolerant) withstands more effectively than cultivar Raj4037 (Salinity susceptible) under salinity. We believe that cultivar Raj-3077 may be very promising to farmers for cultivation in saline areas up to EC 8 dSm-1.

5. Acknowledgement
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6. References
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