Impact of salt stress on five varieties of Wheat (*Triticum aestivum* L.) cultivars under laboratory condition

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**ABSTRACT:** The impact of salt stress under different salinity level (0.25, 50, 75, 100, 125, 150 mM NaCl) on five varieties of wheat viz., HOW-234, HD-2689, RAJ-4101, RAJ-4123, and HD-2045 was conducted. The data showed that different level of salinity significantly affected the growth attributes by reducing root and shoot length for salinity below 125mM. Fresh weight and dry weight of root and shoot were significantly reduced with subsequent treatment. Regarding germination maximum germination was found in variety HD2689 in all the treatments and maximum inhibition was found to be in case of HOW234 variety at 150mM salinity level. Regarding biochemical analysis the sugar, proline content increased with increasing salinity level whereas protein content decreased in the physiologically active leaves of different treatments for all the varieties of wheat. @ JASEM

Wheat is a major cereal crop in many parts of the world and it is commonly known as king of cereals. It belongs to poaceae family and globally after maize wheat is the second most produced food among the cereal crops, rice ranks third. High substrate salinity is a major limiting factor for plants in coastal habitats that germination being one of the most critical periods in life cycle of halophytes (Gilles et.al., 2001; Rubio-Casal et.al., 2003). Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species (Ungar, 2005; Gul et. al., 1999). It was reported that maximum germination of the seeds of halophytic plants occurred in distilled water or under reduced salinity (Gul et. al.,1999; Khan et.al., 2003) and it has been found that germination percentage was reduced with a high NaCl concentrations (Tobe et.al., 2001; Pujol et al., 2000 ; Rubio-Casal et.al., 2003). Excess salinity with the plant root zone has a deleterious effect on plant growth and 8% germination at 1027mol/l level was reported by some workers (Mooring et al.,1971). High level of salinity significantly reduced pigment content in leaves (Al-Sobhi et. al., 2006). According to a report that seed germination of Suaeda salsa seeds decreased significantly with increased salinity. Subsequently it was observed that at low levels of salinity (0.05-0.1mol/l NaCl) seedling growth was increased, while high levels (>0.2mol/l NaCl) inhibited significantly and under salt stress, the leaves accumulated high levels of proline, whereas their soluble sugar content decreased with an increase in salinity level (Duan et al.,2007). The effect of salinity on plants may cause disturbances in plant metabolism (El-Tayeb, 2005). Salinity induced oxidative stress could be a reason for germination inhibition (Amor et al., 2005). Adaptation of plants to salt stress (i.e., resumption of growth after exposure to high soil salinity) requires cellular ion homeostasis involving net intracellular Na⁺ and Cl⁻ uptake and subsequent vacuolar compartmentalization without toxic ion accumulation in the cytosol (Niu et al., 1995; Blumwald et al., 2000; Hasaewaga et al., 1980; Gaxiola et al.,2001). Therefore the aim of the present study were i) to assess the impact of salt stress on different varieties of wheat ii) to screen out best salinity resistant wheat variety and iii) to assess the various biochemical and morphological changes associated with the plants under different salinity gradient.

**MATERIALS AND METHODS**

Seeds were collected in November 2007 from Field Crop Research Station, Department of Agriculture, Government of West Bengal. Five varieties of wheat included HOW-234, HD-2689, RAJ-4101, RAJ-4123, and HD- 2045. Seeds were sterilized by 0.1% HgCl₂ for 30seconds and then washed with fresh water, followed by distilled water. Germination trials were carried out in sterilized petridishes containing a sheet of blotting paper, and thin layer of cotton and moistened with distilled water or saline solution (25, 50, 75, 100, 125, 150mM NaCl). Each of the three replicates contained 10seeds. Each treatment was carried out for 12 days. Germinated seeds were counted, then these seeds were removed from petridishes. Seeds were considered germinating with the emergence of the radicle. Three parameters of germination were determined which includes i) final germination percentage ii) Germination rate: is a measure of rapidity of germination, with lower values indicating faster germination. It is calculated as follows : GR = (n₁) / t₁ + (n₂) / t₂ + ………………… + (nₙ) / tₙ / X where n₁ is the number of germinants at the first day of germination , t₁ is the days from start to fist germination , and Xₙ is the total number of seeds germinated (Rubio-Casal et al.,2003) iii) Mean Daily Germination MDG = (Final germination percentage / number of days to final germination) (Rubio-Casal et al., 2003) Regarding
morphological studies root length, shoot length, fresh weight, dry weight were measured. For biochemical study total chlorophyll (Aron, 1949), total soluble sugar (Mc.Cready et al., 1950), Protein (Lowry et al., 1952) and Proline (Bates et al., 1973) were analysed from physiologically active leaf from different treatments. Statistical analysis including Analysis of variance (ANOVA), Duncan’s multiple range test (DMRT) was performed to study the significance of different salinity gradient on different parameters studied. (Gomez et al., 1984).

### Table 1: Effect of salt stress on HOW-234 wheat cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (mM)</th>
<th>Germination Rate</th>
<th>Mean Daily Germination Rate (MDG)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Root weight (g)</th>
<th>Fresh weight (g)</th>
<th>Dry Weight (g)</th>
<th>Sugar (mg/g)</th>
<th>Protein (mg/g)</th>
<th>Total Chlorophyll (mg/g fw)</th>
<th>Proline mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>90b</td>
<td>0.55abc</td>
<td>7.5ab</td>
<td>13b</td>
<td>14.7a</td>
<td>0.673a</td>
<td>0.088a</td>
<td>0.0082a</td>
<td>0.0009a</td>
<td>0.829a</td>
<td>0.0001a</td>
</tr>
<tr>
<td>NaCl</td>
<td>25</td>
<td>80d</td>
<td>0.65ab</td>
<td>6.80de</td>
<td>14.3a</td>
<td>9.2b</td>
<td>0.574b</td>
<td>0.084b</td>
<td>0.0108b</td>
<td>0.0008b</td>
<td>0.812b</td>
<td>0.0002d</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>95c</td>
<td>0.67a</td>
<td>7.78ad</td>
<td>8.2c</td>
<td>4.4c</td>
<td>0.342c</td>
<td>0.056c</td>
<td>0.0152c</td>
<td>0.0006c</td>
<td>0.678c</td>
<td>0.0004c</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>85c</td>
<td>0.536a</td>
<td>6.94d</td>
<td>7d</td>
<td>3.1d</td>
<td>0.313d</td>
<td>0.051d</td>
<td>0.0161d</td>
<td>0.0044d</td>
<td>0.417d</td>
<td>0.0008b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>90b</td>
<td>0.276e</td>
<td>7.36bc</td>
<td>1.4e</td>
<td>1.8e</td>
<td>0.088e</td>
<td>0.017e</td>
<td>0.017e</td>
<td>0.003e</td>
<td>0.268e</td>
<td>0.0010a</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>65c</td>
<td>0.236f</td>
<td>5.28f</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
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<td>0f</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
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<td></td>
<td>150</td>
<td>6c</td>
<td>0.131</td>
<td>0.049</td>
<td>0.008</td>
<td>0.005</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0006</td>
<td>0.0006</td>
<td>0.0006</td>
<td>4.69X10^-6</td>
</tr>
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</table>

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan’s multiple range test (DMRT)

### Table 2: Effect of Salt Stress on RAJ-4123 wheat cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (mM)</th>
<th>Germination Rate (%)</th>
<th>Mean Daily Germination Rate (MDG)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Root weight (g)</th>
<th>Fresh weight (g)</th>
<th>Dry Weight (g)</th>
<th>Sugar (mg/g)</th>
<th>Protein (mg/g)</th>
<th>Total Chlorophyll (mg/g fw)</th>
<th>Proline mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>100a</td>
<td>1.0a</td>
<td>8.33a</td>
<td>15.6b</td>
<td>14a</td>
<td>0.981a</td>
<td>0.142a</td>
<td>0.0114a</td>
<td>0.0006</td>
<td>0.284a</td>
<td>0.0002e</td>
</tr>
<tr>
<td>NaCl</td>
<td>25</td>
<td>90b</td>
<td>0.95b</td>
<td>7.36b</td>
<td>18.2a</td>
<td>12.9b</td>
<td>0.871b</td>
<td>0.123b</td>
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<td>0.0005</td>
<td>0.139b</td>
<td>0.0006d</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>90c</td>
<td>0.876c</td>
<td>6.53bc</td>
<td>9.7c</td>
<td>5.7c</td>
<td>0.390c</td>
<td>0.065ac</td>
<td>0.0115bc</td>
<td>0.0004</td>
<td>0.220c</td>
<td>0.0009c</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>85cd</td>
<td>0.68d</td>
<td>6.943bc</td>
<td>8c</td>
<td>3.7d</td>
<td>0.351cd</td>
<td>0.057c</td>
<td>0.0202ab</td>
<td>0.0001</td>
<td>0.272b</td>
<td>0.0011b</td>
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<tr>
<td></td>
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<td>75ce</td>
<td>0.63e</td>
<td>6.11e</td>
<td>7.9de</td>
<td>3.5de</td>
<td>0.341de</td>
<td>0.048c</td>
<td>0.0211c</td>
<td>0.00008</td>
<td>0.174d</td>
<td>0.0015a</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>75cef</td>
<td>0.4f</td>
<td>6.11f</td>
<td>2.5g</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
<td>-</td>
<td>0f</td>
</tr>
<tr>
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<td>150</td>
<td>35f</td>
<td>0.18g</td>
<td>0.014</td>
<td>0.130</td>
<td>0.064</td>
<td>0.0050</td>
<td>0.0004</td>
<td>0.0005</td>
<td>5.68X10^-7</td>
<td>6.86X10^-7</td>
<td>0.0004    6.99X10^-6</td>
</tr>
</tbody>
</table>

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan’s multiple range test (DMRT)

### Table 3: Effect of Salt Stress on HD-2689 wheat cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (mM)</th>
<th>Germination Rate (%)</th>
<th>Mean Daily Germination Rate (MDG)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Root weight (g)</th>
<th>Fresh weight (g)</th>
<th>Dry Weight (g)</th>
<th>Sugar (mg/g)</th>
<th>Protein (mg/g)</th>
<th>Total Chlorophyll (mg/g fw)</th>
<th>Proline mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>100a</td>
<td>1a</td>
<td>8.193a</td>
<td>7.36d</td>
<td>14.1a</td>
<td>0.325a</td>
<td>0.091a</td>
<td>0.0067</td>
<td>0.0007b</td>
<td>0.164b</td>
<td>0.001e</td>
</tr>
<tr>
<td>NaCl</td>
<td>25</td>
<td>90e</td>
<td>0.95abc</td>
<td>7.36d</td>
<td>3.5e</td>
<td>13.3b</td>
<td>0.251b</td>
<td>0.086a</td>
<td>0.0105</td>
<td>0.0004c</td>
<td>0.109d</td>
<td>0.0011d</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>95d</td>
<td>0.95abd</td>
<td>7.78abc</td>
<td>9.9b</td>
<td>7.7e</td>
<td>0.171c</td>
<td>0.067ac</td>
<td>0.127b</td>
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<td>0.346a</td>
<td>0.0013c</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100ab</td>
<td>0.95abe</td>
<td>8.193a</td>
<td>8.4d</td>
<td>3.7de</td>
<td>0.074d</td>
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<td>0.001e</td>
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<td>0.0016b</td>
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<tr>
<td></td>
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<td>100abc</td>
<td>0.95abe</td>
<td>8.193a</td>
<td>8.5c</td>
<td>3.9d</td>
<td>0.056e</td>
<td>0.056aec</td>
<td>0.024</td>
<td>0.0008a</td>
<td>0.037e</td>
<td>0.0019a</td>
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<tr>
<td></td>
<td>125</td>
<td>90ef</td>
<td>0.89abf</td>
<td>7.36def</td>
<td>1.4f</td>
<td>1.1f</td>
<td>0.017f</td>
<td>0.021de</td>
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<td>0f</td>
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<tr>
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<td>150</td>
<td>80g</td>
<td>0.816ahg</td>
<td>6.806e</td>
<td>1.2f</td>
<td>0.9f</td>
<td>0.012f</td>
<td>0.005g</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
<td>0f</td>
</tr>
</tbody>
</table>

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan’s multiple range test (DMRT)

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RESULTS AND DISCUSSION

Germination percentage of wheat cultivars was significantly affected by the salt stress (p<0.05). Germination percentage was reduced from 125mM NaCl salt concentration onwards for almost all the varieties. Seed germination was found to be highest in distilled water or RAJ-4123 variety. Rate of germination the results were significant (p<0.05) for all the varieties. The reduced level of seed germination may be due to (i) loss of viability at higher salinity level (ii) delaying germination of shoot elongation due to slowing down the water uptake. The levels of salinity may have also inhibit the root and shoot development may be due to toxic effects of the solution. The reduction in root and shoot length as roots are directly exposed to salt solution. The effect of salt stress was completely inhibitory at 125mM and 150 mM NaCl concentration .Increase in the salinity from 0 to 25mM NaCl had no effect on plant root and shoot length , while further increase from 50mM onwards significantly reduced the root length and shoot length . The effect of salt stress was completely inhibitory at 125mM and 150 mM NaCl concentrations for almost all the varieties except HD-6859 wheat cultivar. Growth processes are specially sensitive to the effects of salt, so that growth rates and biomass production provide reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it as reported by Amor et al., 2005). In the current investigation the higher level of salinity has a more pronounced effect on root length with respect to shoot length as roots are directly exposed to salt solution. The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake.

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uptake for overall osmotic adjustments of the plant body under high salt stress condition.

Regarding fresh weight and dry weight the effect of salt stress was pronounced from 50mM NaCl concentration onwards. It was completely inhibitory from 75mM onwards for RAJ – 4101. The proportion of fresh weight and dry weight allocated to root, shoot and leaf (whole plant body) decreased with increased NaCl levels.

Compared to the control, the salt stress affected wheat plants showed higher accumulation of total soluble carbohydrate from 25mM NaCl concentrations onwards up to 100mM. There was a decreasing trend of protein content of leaves with subsequent increase in the salt concentrations as compared to the control. The salt stress significantly affected (p<0.05) affected the chlorophyll synthesis in plants from 75mM onwards up to 100mM. There was gradual reduction in total chlorophyll accumulation in the leaves as compared to control or all the varieties in the present investigation. Enhanced level of proline accumulation was found to be in case of plants under treatments of higher concentration of salt solution (NaCl) from 25mM onwards to 100 mM. Compared to the control (without NaCl salt treatment) the wheat cultivars showed higher content of total soluble sugars.

Accumulation of sugars in response to abiotic stress (PEG-induced water stress) has been reported earlier by several workers (Handa et al., 1980; Srivastava et al., 1996). The sugars might contribute to salt stress tolerance either by serving as osmoticum or as respiratory substrates. In addition, high level of carbohydrate status favoured proline accumulation (Hanson, 1982). The reduced level of protein in the physiologically active leaves is due to reduced capacity to incorporate amino acids into proteins and an increase in proteolytic enzymes or due to contribution of polysomes to monosomes under stress condition or due to synthesis of abscisic acid increases the activity of RNase, thus indirectly inhibiting the protein synthesis as reported by Singh et al., 1985. Moreover release of proteins under stress condition by the stressed cells of the plants involved in nutrient transport and other protein released were an important component for membrane structure providing cells the ability to withstand high degree of salt stress as reported by Hasegawa et al., 2000 and Handa et al., 1983. The reduced level of total chlorophyll content under high salt stress condition in the leaves which may be due to membrane deterioration of the cell membrane of the chloroplastid leading towards lesser accumulation of chlorophyll and lesser photosynthetic efficiency as reported by several workers (Seeman et al., 1985). Enhanced level of proline in the leaves of the salt treated plants may be due to fresh synthesis or from breakdown of proline rich proteins during stress as has been reported by several other earlier works (Greenway et al., 1980) which may be an adaptive role of proline accumulation in relation to survival, rather than maintained growth, since it only accumulated when growth inhibition was already pronounced. From the current investigation it can be concluded that root growth was found to be highly sensitive parameter and the varieties HD-2689, Raj4123 and HD-2045 seem to be relatively tolerant with respect to salt stress than other cultivars under study.

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