ANTIOXIDATIVE RESPONSE OF MUNGBEAN
(VIGNA RADIATA L.) TO SALT STRESS

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ABSTRACT
The effect of salt stress (NaCl) on growth, specific activity of catalase and peroxidase and accumulation of proline and Na⁺ and K⁺ content in leaves from mungbean were examined. The plant dry weight decreased with higher concentrations of the salt. The results at the various growth stages revealed an increase in the activity of catalase and peroxidase activity with the increasing intensity of salt stress from 50 mM to 200 mM NaCl. Activity of catalase and peroxidase significantly increased at 20 days after sowing (DAS) as compared to control. The proline accumulation also increased with higher concentration of salt, whereas K⁺ concentration decreased that caused an injury to the plants.

INTRODUCTION
Plant cells have to cope constantly with the dangers produced by the oxygen radicals, and as a protective system they have evolved a complex series of enzymatic and non-enzymatic antioxidant mechanisms (Del Rio et al., 1991). In plants, the production of the active oxygen species have been evidenced during photo-oxidative processes induced during abiotic stress conditions such as chilling, salinity, ozone etc. (Foyer and Mullineaux, 1994). Biochemical studies have identified over reduction of photosystem II, the Mehler reaction and photorespiration as potentially important sources of the active oxygen species (Foyer, 1996). During these mechanisms, oxygen is monovalently reduced to superoxide (O₂⁻) and then to H₂O₂. This H₂O₂ is subsequently converted to water by catalase and peroxidase by two different mechanisms. During osmotic stress, plants induce processes that regulate the osmotic adjustment to maintain sufficient cell turgor for growth to continue. Such adjustment requires the control of intercellular inorganic and organic compounds compartmented mainly in the cytoplasm. These solutes termed as osmolytes raise osmotic pressure and protect some macromolecules against denaturation. Proline is the most diversely nitrogenous compound accumulated under osmotic stress in plants, paying a major role in osmotic adjustment (Aziz et al., 1999).

MATERIAL AND METHODS
Plant material, growth conditions and salt treatment
Seeds of mungbean (Vigna radiata L. variety, HUM-1) were obtained from the Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi (India). Seeds were surface sterilized with sodium hypochlorite (1%) and sown on July 02 directly in pots (size) filled with soil (3kg). After germination, only five seedlings were left in each pot. Plants were grown in a greenhouse with a 16/8h photoperiod at 25°C/21°C and 55/75% RH (day/ night). Salt stress (50, 100 and 200 mM NaCl) was imposed in one osmotic shock to 5 days old seedlings. Experiment was replicated thrice to reduce the error to minimum. All the observations were recorded at 20 and 40 days after sowing.

Extraction and assay of catalase and peroxidase activity
Weighed samples were ground in a pre-chilled pestle and mortar in 5mL of ice cold 0.1M Tris-HCl buffer containing 5x10⁻³ M 2-mercaptoethanol. The extract was centrifuged at 10, 000 rpm for 25 minutes at 4°C. The supernatant was used for the assay
of catalase and peroxidase activity. In case of catalase, protein content was determined using a dye binding method as proposed by Clairborne, (1985) and one unit of catalase activity represents the breakdown of 1mmole \( \text{H}_2\text{O}_2 \) min\(^{-1}\). Peroxidase activity was determined specifically with guaicol at 470nm (extraction coefficient 25.2 nm cm\(^{-1}\)) following the method proposed by Polle et al. (1994). The reaction mixture contained 100mM potassium phosphate buffer (pH 6.5), 16mM guaicol and 10mM of 10% \( \text{H}_2\text{O}_2 \) in 3mL volume. The reaction was initiated by adding plant extract for 10 minutes.

**Proline estimation**

Free proline was determined by the ninhydrin assay (Bates et al., 1973).

**\( \text{Na}^+ \) and \( \text{K}^+ \) estimation**

\( \text{Na}^+ \) and \( \text{K}^+ \) were determined in plant samples by flame photometry as detailed by Tandon (1995).

**Statistics**

The data was statistically analyzed using analysis of variance (ANOVA) to compare the means. The individual treatment was assessed by computation of least significant difference taking \( t \) values for error d.f. at 5% level of significance.

**RESULTS AND DISCUSSION**

**Growth response to salt stress**

Nine days after the application, salt burning at the leaf edges was observed causing slight marginal chlorosis. In terms of growth measured as dry weight, the inhibition due to salt stress was quite evident during the first two weeks. The dry weight was found to decrease, as the concentration of salt increased in the growing medium from 50-200mM (Table 1). The dry weights in such plants decreased 26.25 per cent at 20 days after sowing (DAS) and 44.8 per cent at 40 DAS as reported earlier also West and Francios (1982). Growth inhibition is associated with the reduction of endogenous levels of polyamines. The decrease in the level of polyamines was noted to be related to the cellular accumulation of \( \text{Na}^+ \) and to leakage of \( \text{K}^+ \) (Aziz et al., 1999). In this experiment too the \( \text{Na}^+ \) level increased significantly in contrast to a significant decrease in the level of \( \text{K}^+ \) (Table 3).

**Catalase and Peroxidase activity and Proline content**

Salinity caused significant increase in the activity of catalase and peroxidase (Table 2). The activity of catalase and peroxidase increased by 2.4 and 2.8 times respectively at a concentration of 200mM \( \text{NaCl} \) at 20 DAS. This increase has also been reported by earlier workers (Djnauguiraman et al., 2004). The increase in the activity of antioxidative enzymes (catalase and peroxidase) may be due to the production of higher levels of active oxygen species (AOS) during the salt stress. The antioxident character of catalase and peroxidase pertains to their capacity to remove \( \text{H}_2\text{O}_2 \) formed during stress. Catalase has been found to remove the bulk of \( \text{H}_2\text{O}_2 \), whereas the peroxidase would be involved in the scavenging of \( \text{H}_2\text{O}_2 \) that is not removed by the catalase (Willekens et al., 1997).

The level of proline was positively affected by all the concentrations of \( \text{NaCl} \) at 20 and 40 DAS of the treatment (Table 2). During the period of stress, proline level was increased by 70.4 and 73.4 per cent at 200mM \( \text{NaCl} \) as compared to plants growing under salt free conditions at 20 and 40 DAS. Salt induced increase in proline concentration started shortly after the salt stress application. The observation is in agreement with the results obtained by Trichant et al. (2004). It is reasonable to believe that proline could contribute to maintain the turgor pressure essential for continued growth during salinization. Besides the biophysical effects of proline as an osmo-compatible solute, its biosynthesis can contribute to reduced cellular acidification allowing the regeneration of NADP\(^+\) needed for maintenance of respiration...
Table 1. Response of dry weight (mg) and sodium (µmole g⁻¹ DW) and K content (µmole g⁻¹ DW) of mungbean to salt stress at two growth stages

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight</th>
<th>20DAS</th>
<th>40DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.80</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.75</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.61</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.59</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>2.6</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Activity of catalase (µgH₂O₂/min/g), peroxidase (µMmin⁻¹mg protein) and proline (nmole g⁻¹ FW) content at two growth stages as affected by varying levels of NaCl in mungbean

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Catalase</th>
<th>Peroxidase</th>
<th>Proline</th>
</tr>
</thead>
<tbody>
<tr>
<td>20DAS</td>
<td>40DAS</td>
<td>20DAS</td>
<td>40DAS</td>
</tr>
<tr>
<td>0</td>
<td>107.2</td>
<td>109.3</td>
<td>3.92</td>
</tr>
<tr>
<td>50</td>
<td>110.8</td>
<td>121.5</td>
<td>7.01</td>
</tr>
<tr>
<td>100</td>
<td>208.8</td>
<td>228.5</td>
<td>9.23</td>
</tr>
<tr>
<td>200</td>
<td>261.4</td>
<td>273.7</td>
<td>11.17</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>2.5</td>
<td>3.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 3. Effect of NaCl on sodium content (µmole g⁻¹ DW) and K content (µmole g⁻¹ DW) of mungbean at different stages of growth

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium content</th>
<th>Potassium content</th>
</tr>
</thead>
<tbody>
<tr>
<td>20DAS</td>
<td>40DAS</td>
<td>20DAS</td>
</tr>
<tr>
<td>0</td>
<td>30.5</td>
<td>43.2</td>
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<tr>
<td>50</td>
<td>70.3</td>
<td>109.7</td>
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<tr>
<td>100</td>
<td>80.4</td>
<td>183.4</td>
</tr>
<tr>
<td>200</td>
<td>106.3</td>
<td>239.8</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>2.5</td>
<td>3.8</td>
</tr>
</tbody>
</table>

and photosynthesis processes (Larher et al., 1998). Proline itself might have served as nitrogen and carbon sources needed in stress recovery in the present experiment (Guistino et al., 2004) and might have acted as a scavenger of hydroxyl radicals avoiding cellular damage provoked by osmotic or salt induced oxidative stress (Borsani et al., 1999).

Accumulation of Na⁺ and K⁺

After 20 and 40 days of stress at 200mM NaCl, the increase in intracellular Na⁺ was 3.5 and 7.4 fold respectively in comparison to control plants (Table 3). However, the accumulation of K⁺ in shoots revealed the opposite trend in contrast to Na⁺. A gradual decline in K⁺ content was noticed with increasing levels of salinity (Table 3). The control plants observed the highest content of K⁺, whereas the salt treated plants exhibited a decrease in K⁺. At the highest concentration of the NaCl (200mM), the K⁺ was reduced by 68 and 71 per cent after 20 and 40 DAS respectively as compared to the unstressed seedlings (control). The salt sensitivity of mungbean could also be attributed to the decrease in leaf K⁺ with time of exposure, indicating its inability to maintain K⁺ selectivity (Perez-Alfocea et al., 1993), thus, the leaf K⁺ level started to decrease when the Na⁺ content increased significantly relative to control.

Overall, the findings as discussed above reveal that an efficient defense mechanism may be involved in the increase of antioxidant levels in terms of catalase and
peroxidase activities coupled with significant damaging action of the salt stress. Proline accumulation that prevents the

REFERENCES