Stress responses in two genotypes of mulberry (*Morus alba* L.) under NaCl salinity

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Changes in biomass yield rates, cell membrane stability (CMS), malondialdehyde (MDA) content and in the levels of physiological stress markers such as proline and glycine betaine in two high yielding genotypes (S1) and ATP, salt tolerant and salt sensitive, respectively of mulberry under NaCl salinity were studied. Biomass yield rates and CMS were significantly decreased in both the genotypes under stress conditions. Per cent of decrease in biomass yield rate and CMS was relatively less in S1 than in ATP. Salt stress results a significant increase in the accumulation of proline, by 6-fold in S1 and 4-fold in ATP. Glycine betaine content was also increased significantly in stressed plants. However, the per cent increase was more in S1 than in ATP. The level of lipid peroxidation as indicated by MDA formation was greater in ATP than in S1. These results clearly support the better salt tolerant nature of S1 compared to ATP genotype.

Salinity mainly occurs in arid and semi-arid conditions where the precipitation is not enough to leach the excess soluble salts from the root zone. Salinity reduces the value and productivty of the affected land. Salinity stress is a major factor in limiting crop productivity throughout the world and has recently been the focus of much research. An understanding of physiological mechanisms and identification of specific physiological traits conferring salt tolerance could play a major role in the development of breeding strategies for transferring the higher levels of salinity tolerance from wild relatives to the cultivated ones. Defining salt tolerance, however, is quite difficult because of the complex nature of salt stress and the wide range of plant responses.

In higher plants and other organisms, a common metabolic adaptation to salinity or drought stress is the accumulation of osmoprotectants. Osmoprotectants are small molecules that can benifit osmotically stressed cells in two ways—by acting as nontoxic cytoplasmic osmolytes to raise osmotic pressure, and by stabilizing enzymes and membranes against damage by high salt levels. Osmoprotectants fall into two chemical classes: Polyols and their derivatives, and small zwitter-ions such as amino acids and betaines. By lowering water potentials, the accumulation of compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism. Alterations in the dry mass accumulation under salt stress was studied by several workers. In agricultural studies the productivty of crops in terms of biomass production can be a key parameter to evaluate salinity effects. MDA which is one of the decomposition product of polyunsaturated fatty acids (PUFA) of biomembranes showed greater accumulation under salinity treatment. Cell membrane stability test has been widely used to differentiate stress tolerant and susceptible cultivars of many crops and in some cases higher membrane stability could be correlated with better field performance.

Information regarding the relative levels of salt tolerance among mulberry varieties is lacking, therefore in the present study an attempt is made to assess the tolerance potentials in mulberry cultivars with different sensitivity to salt stress based on physiological stress markers and tolerant indices.

Mulberry (*Morus alba* L.) cultivars S1 and ATP were procured from Regional Sericultural Research Station (CSB), Anantapur, India. The cuttings of approximately equal length and diameter, with 3 to 4 active buds were prepared and immediately planted in earthen pots containing 8 kg of red loamy soil and farm yard manure (3:1 ratio). The pots were watered daily. The pots were kept in the Botanical Garden of the institute under natural photoperiod of 12-13 hr and temperature of 32±4°C. Three-month-old plants
were subjected to salt stress induced by a range of NaCl concentrations (0.0, 0.5, 1.0 and 1.5%) and maintained for a period of 12 days. The electrical conductivity (EC) of soil saturation extract was 1.7, 2.0, 4.0 and 5.9 mhos/cm², respectively. Care was taken to avoid drainage of soil solution during the treatment by giving water slightly less than field capacity. EC of soil extract was monitored and adjusted on alternate days.

The plants were washed with deionised water and blotted dry with filter paper. Leaf parts were separated and fresh weights were taken. The material was dried at 80°C in a hot air oven for 48 hr and dry weights were recorded. Cell membrane stability measured according to the method of Premachandra et al. and was calculated as per cent injury from the following equation.

\[
\% \text{ injury} = 1 - \frac{(1 - T1/T2)}{(1 - C1/C2)} \times 100,
\]

where T1 and T2 are first and second conductivity measurements of the salt treatment C1 and C2 are first and second conductivity measurements of the control.

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA), a product of lipid peroxidation, by thiobarbituric acid (TBA) reaction described by Puever and Higgins. Quaternary ammonium compounds were measured in terms of glycine betaine equivalents according to Grieve and Grattan. Free proline content was extracted in the aqueous sOlnphosalycylic acid and estimated according to Bates et al.

Dry mass accumulation was decreased in salt stressed plants of both genotypes compared to controls. These results are in agreement with reports by several investigators. Magnitude of decline in leaf dry mass accumulation was found to be dependent on severity of stress. However, the per cent inhibition in dry mass yield was relatively lesser in S1 than in ATP (Table 1). Dry weight decreased as a result of stress and attributed to altered carbon and nitrogen metabolism which are responsible for total biomass production. Decreased in dry mass accumulation of leaves also attributed to decreased rates of reduced leaf area, photosynthesis and chlorophyll content.

Proline accumulated significantly more in stressed leaves. These results are in agreement with reports by several investigators. Free proline content was significantly increased in stressed plants when compared to control plants at all levels of stress. However, per cent increase was more in S1 (6-fold) than ATP (4-fold). High level of proline enabled the plant to maintain osmotic phenomenon when growing under low water potentials. In addition to acting as osmoprotectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxy radical scavenger, as a solute that protects macromolecules against denaturation, and as means of reducing the acidity in the cell, proline may also act as a storage compound and nitrogen source for rapid growth after stress. Accumulation of proline in stressed plants is caused both by the activation of proline biosynthesis and a reduced rate of its catabolism has been observed.

Glycine betaine, has been reported as an osmotic solute. In the present study there was significant increase in glycine betaine in leaves of both the varieties. Per cent increase in quaternary ammonium compounds, glycine betaine, accumulation was more in S1 (10-fold) than ATP (6-fold) cultivar. An increase in glycine betaine levels under stress conditions was found to increase the sodium-flux from cytoplasm to vacuole and was also known to modify the membrane behaviour. Glycine betaine is ubiquitous protein-stabilizing osmolyte occuring from bacteria to higher plants and animals. In addition to osmoregulation, it stabilizes the oxygen-evolving activity of photosystem-II protein complexes by protecting against dissociation of regulatory extrinsic proteins and also stabilizes manganese cluster. Both proline and glycine betaine under stress conditions may be able to induce formation of strong H-bonded water around the protein, preserving the native state of the cell biopolymers.

Extent of damage to the membrane was monitored by measuring the amount of thiobarbituric acid reactive material (MDA), produced when polyunsaturated fatty acids in the membrane undergo peroxidation. With increasing level of salinity stress, MDA content increased in the sensitive variety indicating an increase in lipid peroxidation. S1 (tolerant variety), on the other hand, did not exhibit increased in lipid peroxidation with exposure to salinity stress (Table 1). By generating changes in unsaturated fatty acids free radical formation and lipid peroxidation under salt stress in salt sensitive varieties may be enhanced affecting membrane integrity, as evidenced by increase in solute leakage.

Cell membrane stability has been used to assess stress tolerance by several reports. In the present study, a smaller per cent membrane injury was observed in S1 compared to ATP which further
Table 1—Drymass, proline content, glycine betaine content, melondialdehyde content and cell membrane injury in leaves of control and NaCl stressed mulberry varieties on day 12 after inducing stress

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conc. of NaCl (%)</th>
<th>S1</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mass (g⁻¹ plant)</td>
<td>A</td>
<td>5.31 ±0.81</td>
<td>2.9a ±0.91</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4.52 ±0.82</td>
<td>2.23b ±0.19</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.55 ±0.73</td>
<td>1.62c ±0.62</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2.42 ±0.38</td>
<td>0.98d ±0.11</td>
</tr>
<tr>
<td>Proline (µg g⁻¹ fresh wt.)</td>
<td>A</td>
<td>25.6a ±3</td>
<td>23.2a ±0.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>64.07b ±0.9</td>
<td>43.06b ±0.86</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>110.14c ±1.03</td>
<td>62.98c ±3.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>164.02d ±1.32</td>
<td>95.18d ±0.36</td>
</tr>
<tr>
<td>Glycine betaine (m-mole g⁻¹ dry wt.)</td>
<td>A</td>
<td>2.07a ±0.10</td>
<td>1.9a ±0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4.55b ±0.6</td>
<td>2.89b ±0.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>15.13c ±1.0</td>
<td>8.74c ±3.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>22.37d ±1.3</td>
<td>12.56d ±3.9</td>
</tr>
<tr>
<td>MDA (µ mole g⁻¹ dry wt.)</td>
<td>A</td>
<td>0.46a ±0.04</td>
<td>0.52a ±0.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.58b ±0.03</td>
<td>0.58b ±0.08</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.71c ±0.02</td>
<td>0.71c ±0.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.82d ±0.04</td>
<td>0.82d ±0.01</td>
</tr>
<tr>
<td>Cell membrane injury (%)</td>
<td>50 mM</td>
<td>48.76</td>
<td>34.87</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>60.32</td>
<td>89.15</td>
</tr>
<tr>
<td></td>
<td>150 mM</td>
<td>72.31</td>
<td>97.67</td>
</tr>
</tbody>
</table>

The mean values in a column followed by a different letter for each plant species are significantly different (p<0.05) according to Duncan's multiple range (DMR) test.

Figures in parentheses represent per cent of control.

A-Control; B-0.5%; C-1.0% and D-1.5%.

supports the tolerant nature of S1. Lesser electrolyte leakage has been correlated with the greater membrane integrity under stressful conditions and those are characterized as salt stress tolerant genotypes.

Stress markers including free proline and glycine betaine are substantially higher in the variety S1. Salt tolerant nature of S1 has also been evidenced by a relatively lesser level of malondialdehyde. Further, the results of CMS supports the tolerant nature of S1.

From the present study, it is evident that some physiological parameters like free proline content, QAC's and the tolerance indices such as CMS and MDA content may be considered as simple physiological traits to assess the salt tolerant potentials of mulberry genotypes.

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References