INTRODUCTION

Soil salinity represents an increasing threat to agricultural production. High sodium (Na+) concentrations in soil are toxic to higher plants (Demiral and Turkan 2005). Osmotic stress due to drought and salinity is the most serious problem that limits plant growth and crop productivity in agriculture (Zhu et al. 2004). In arid and semi-arid regions, soil salinity is a common occurrence. The use of poor irrigation water and saltwater encroachment is also increasingly threatening agriculture in humid regions (Salekdeh et al. 2002).

Blackgram (Vigna mungo L. Hepper) is an important grain legume crop grown in the tropical and subtropical regions of the Indian subcontinent. It is grown for its protein-rich edible dry seeds that, when supplemented with cereals, provide a balanced diet. The crop is subjected to a variety of abiotic and biotic stresses which are responsible for its poor productivity; among the abiotic stresses the crop is notably susceptible to salinity and drought (Bhomkar et al. 2008).

Salt stress can lead to stomatal closure, which reduces CO2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of reactive oxygen species (ROS) and induce oxidative stress (Parida and Das 2005; Parvaiz and Satyawati 2008). ROS, including superoxide radical (O2•−), hydroxyl radical (OH•), singlet oxygen (1O2) and hydrogen peroxide (H2O2), are also generated naturally via a number of cell metabolic pathways (Kanazawa 2000). Thus, ROS cause constant problems to aerobic organisms. ROS react with cellular components causing significant damage to membranes and other essential macromolecules such as photosynthetic pigments, protein, nucleic acids and lipids (Lin and Kao 2000). Hence the importance of controlling the level of ROS in cells.

ABSTRACT

The effects of triadimefon (TDM), a triazole compound, on NaCl-stressed blackgram (Vigna mungo (L.) Hepper) plants were studied to understand the ameliorative effect of this triazole derivative. Seeds were sown in plastic pots and irrigated with groundwater up to 35 days after sowing (DAS) to field capacity. Later plants were irrigated with groundwater as control and others were treated with 100 mM NaCl, 100 mM NaCl + 20 mg l−1 TDM and 20 mg l−1 TDM. The samples were collected randomly at 40 and 80 DAS. Salinity treatment decreased the protein content and increased the amino acid, proline, glycine betaine (GB), ascorbate peroxidase (APX) and catalase (CAT) activities when compared to NaCl-stressed plants. TDM treatment increased all parameters compared with the control. However, TDM-mediated salinity tolerance could be attributed to the increased activities of APX and CAT when compared to NaCl-stressed plants.

Keywords: ascorbate peroxidase, catalase, glycine betaine, NaCl, proline, salinity amelioration, TDM, triazole, Vigna mungo

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DAS, days after sowing; DW, dry weight; ROS, reactive oxygen species; SOD, superoxide dismutase; TDM, triadimefon

MATERIALS AND METHODS

Plant material

The seeds of blackgram (Vigna mungo (L.) Hepper) were obtained from the Pulses Research Division, Rice Research Institute, Tamil Nadu, India. Seeds were surface sterilized with 0.2% HgCl2. Ten seeds were sown in each pot 30 cm in diameter and 30 cm high
containing 3 kg of soil mixture composed of red soil, sand and farmyard manure (FYM) at a 1:1:1 ratio. All the pots were watered to field capacity with ground water up to 35 DAS. The plants were thinned to 2 plants pot⁻¹ on 35 DAS. Plants were irrigated with groundwater marked as control and other treated with 100 mM NaCl, 100 mM NaCl + 20 mg l⁻¹ TDM and 20 mg l⁻¹ TDM alone, respectively on 35 and 75 DAS. The plants were uprooted randomly on 40 and 80 days after sowing (DAS) and separated into root, stem and leaves and used for determining biochemical parameters and antioxidant enzyme activities.

### Protein and total free amino acid

The protein content was determined by the method of Bradford (1976). Total free amino acid was extracted and estimated by following the method of Moore and Stein (1948).

### Glycine betaine content

The amount of glycine betaine (GB) was estimated according to the method of Grieve and Grattan (1983). The plant tissues (roots, stem and leaves) were finely ground, mechanically shaken with 20 ml deionised water for 24 h at 25°C. The samples were then filtered and filtrates were diluted 1:1 with 2 N H₂SO₄. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I₂ reagent was added and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4°C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0°C. The supernatant was carefully aspirated with a fine glass tube. Periodide crystals were dissolved in 9 ml of 1,2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB (Himedia, Mumbai, India) as standard and expressed in mg g⁻¹ DW.

### Ascorbate peroxidase

Ascorbate peroxidase (EC 1.11.1.1) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂ and 200 μl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H₂O₂ (extinction coefficient 2.9 mM⁻¹ cm⁻¹). The enzyme activity was expressed in U mg⁻¹ protein (U = change in 0.1 absorbance min⁻¹ mg⁻¹ protein).

### Catalase

Catalase (EC 1.11.1.6) was measured according to Chandlee and Scandalios (1984) with modifications. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U mg⁻¹ protein (U = 1 mM of H₂O₂ reduction min⁻¹ mg⁻¹ protein).

### Statistical analyses

The data were subjected to analysis of variance (ANOVA) using the GLM module of Costat (CoHort Software, Monterey, CA, USA). Duncan’s multiple range test was used to separate treatment means in case of a significant F-test at P ≤ 0.05.

### RESULTS AND DISCUSSION

Sodium chloride treatment decreased protein content in roots, stems and leaves of blackgram plants on 40 and 80 DAS when compared with the control (Table 1). A decrease in protein content has been reported in a number of plants like Atriplex halimus (Bajji et al. 1998), radish (Muthukumarasamy et al. 2000) and sorghum (Azooz et al. 2004). The combination of NaCl with TDM increased protein content in all parts of the plant except in roots on 40 DAS when compared with NaCl-stressed plants. Similar results were observed in NaCl-stressed and TDM-treated Vigna unguiculata plants (Gopi et al. 1998). Unstressed plants treated with TDM had higher protein content when compared with control.

Salinity treatment increased the amino acids, proline and GB content of all parts of the plants to a larger extent (Tables 2-4). Similar observations were made in NaCl-treated soybean (Panneerselvam et al. 1998), sorghum (Azooz et al. 2004) and Arachis hypogaea plants (Girija et al. 2002). Treatment with TDM on NaCl-stressed plants markedly reduced the accumulation of amino acids, proline and GB content when compared with NaCl-stressed plants. However, NaCl-stressed plants had a higher content of amino acids, proline and GB than the control. These results confirm the findings of Saha and Gupta (1993) who found an increase in the content of amino acids in salt-stressed Vigna unguiculata seedlings treated with TDM. However, TDM treatment increased amino acid, proline and GB con-

### Table 1

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Sampling days</th>
<th>Control 100 mM NaCl</th>
<th>100 mM NaCl + 20 mg L⁻¹ TDM</th>
<th>20 mg L⁻¹ TDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>40</td>
<td>82.36 ± 2.51 a</td>
<td>83.29 ± 2.55 a</td>
<td>83.47 ± 2.50 a</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>77.23 ± 1.89 a</td>
<td>80.21 ± 2.51 c</td>
<td>78.01 ± 1.88 a</td>
</tr>
<tr>
<td>Stem</td>
<td>40</td>
<td>63.14 ± 1.45 a</td>
<td>66.55 ± 1.41 c</td>
<td>63.35 ± 1.44 a</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>58.36 ± 1.03 a</td>
<td>62.31 ± 1.40 c</td>
<td>59.13 ± 1.05 a</td>
</tr>
<tr>
<td>Leaf</td>
<td>40</td>
<td>74.16 ± 1.78 a</td>
<td>79.30 ± 1.66 c</td>
<td>77.38 ± 1.43 d</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>72.64 ± 1.23 a</td>
<td>77.51 ± 1.71 b</td>
<td>74.62 ± 1.77 c</td>
</tr>
</tbody>
</table>

Values are expressed in mg g⁻¹ dry weight. Values are given as mean ± SD of six samples in each group. Values that do not share a common letter differ significantly at P ≤ 0.05 (DMRT).

### Table 2

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Sampling days</th>
<th>Control 100 mM NaCl</th>
<th>100 mM NaCl + 20 mg L⁻¹ TDM</th>
<th>20 mg L⁻¹ TDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>40</td>
<td>176.6 ± 6.08 a</td>
<td>182.9 ± 6.51 b</td>
<td>182.5 ± 6.23 c</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>169.2 ± 5.51 a</td>
<td>182.6 ± 6.20 b</td>
<td>174.2 ± 6.52 c</td>
</tr>
<tr>
<td>Stem</td>
<td>40</td>
<td>165.1 ± 5.41 a</td>
<td>171.4 ± 6.48 c</td>
<td>171.5 ± 5.90 c</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>158.4 ± 4.34 a</td>
<td>163.8 ± 5.22 c</td>
<td>164.6 ± 5.40 c</td>
</tr>
<tr>
<td>Leaf</td>
<td>40</td>
<td>191.4 ± 6.52 a</td>
<td>197.2 ± 6.87 c</td>
<td>197.2 ± 6.88 c</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>184.3 ± 6.31 a</td>
<td>190.9 ± 6.56 c</td>
<td>190.8 ± 6.53 d</td>
</tr>
</tbody>
</table>

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tent more than the control. Similar effects of TDM have already been reported in radish (Muthukumarasamy et al. 2000), cucumber seedlings (Feng et al. 2003) and wheat seedlings (Berova et al. 2002).

Salt stress increased APX activity in blackgram plants compared with the control (Table 5). The higher level of endogenous APX activity is essential to maintain the antioxidant system that protects plants from oxidative damage due to biotic and abiotic stresses (Shigeoka et al. 2000). The H2O2 scavenging systems represented by APX and CAT activities were more important in imparting tolerance in wheat varieties (Sairam et al. 1995; Berova et al. 2002).

Salinity increased CAT activity in all parts of blackgram plants compared with the control (Table 6). Panda (2001) reported that salt stress decreased CAT activity in greengram. Application of TDM to NaCl-stressed plants resulted in increased CAT activity compared with NaCl-stressed plants. However, TDM treatment alone also increased CAT activity when compared with the control. Similar results were observed in TDM-treated Catharanthus roseus plants (Jaleel et al. 2006). The H2O2 scavenging systems represented by APX and CAT activities were more important in imparting tolerance in wheat varieties (Sairam et al. 1998).

It can be concluded that TDM treatment induced an increase in protein content in NaCl-stressed plants and might be the reason for decreased amino acid, proline and GB content when compared with NaCl-stressed plants. However, TDM-mediated salinity tolerance could be attributed to the increased activities of APX and CAT when compared to NaCl-stressed plants.

REFERENCES


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Salt stress increased APX activity in blackgram plants compared with the control (Table 5). The higher level of endogenous APX activity is essential to maintain the antioxidant system that protects plants from oxidative damage due to biotic and abiotic stresses (Shigeoka et al. 2002). Application of TDM increased APX activity when compared with NaCl-stressed plants. Moreover, TDM treatment alone also increased APX activity when compared with the control and other treatments. In experiments with another triazole compound, paclobutrazol, there was increased level of APX activity in wheat cultivars (Kraus et al. 1995; Berova et al. 2002).

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