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Triadimefon Protects Blackgram (*Vigna mungo* L. Hepper) Plants from Sodium Chloride Stress

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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 in blackgram compared with the control. The addition of NaCl with TDM showed an increase in protein, APX and CAT activities and decreased proline, GB content when compared with 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

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 CO_2 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 (O^{2-}), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), 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.

For effective detoxification of ROS, plants possess various protective antioxidant mechanisms, including antioxidant molecules like ascorbic acid, α-tocopherol, reduced glutathione and antioxidant enzymes like ascorbate peroxidase (APX), superoxide dismutase (SOD) and CAT (Prochazkova *et al.* 2001). SOD catalyses the dismutation of superoxide to H_2O_2 and O_2, H_2O_2 is further detoxified by catalase (CAT) to water and oxygen (Zhu *et al.* 2004). Salinity causes an excessive generation of ROS in plants (Foyer *et al.* 1994). The ascorbate-glutathione cycle has been shown to be of great importance in multiple stress reactions (Drazkiewicz *et al.* 2003). Therefore, the interactions between production and scavenging of ROS should be balanced to maintain the plants in a relatively stable state (Lin and Kao 2000).

The triazole derivatives are fungitoxic and with plant growth regulating properties and are reported to change the balance of important plant growth regulators, including gibberellins, abscisic acid and cytokinins (Fletcher *et al.* 2000). Apart from these plant growth regulating properties, triazoles protect plants from various types of environmental stresses (Fletcher and Hofstra 1990) like drought, low and high temperatures, UV-B radiation, air pollutants and fungal pathogens, thus making it a plant multi protectant (Voesenek *et al.* 2003). In the present study we determined whether TDM could protect blackgram plants subjected to salinity stress.

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% $HgCl_2$. Ten seeds were sown in each pot 30 cm in diameter and 30 cm high

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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 H_2SO_4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 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.

Proline content

The proline content was estimated by the method of Bates *et al*. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. Supernatant was used for estimation of proline content. The reaction mixture consisted of 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm.

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 H_2O_2 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 H_2O_2 (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 H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 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 $P \leq 0.05$.

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 Effect of salt stress with or without TDM on protein content of different parts of *Vigna mungo* plants on different sampling days.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	82.36 ± 2.51 a	78.52 ± 1.32 b	83.29 ± 2.55 a	83.47 ± 2.50 a
	80	77.23 ± 1.89 a	74.25 ± 1.25 b	80.21 ± 2.51 c	78.01 ± 1.88 a
Stem	40	63.14 ± 1.45 a	59.51 ± 1.12 b	66.55 ± 1.41 c	63.35 ± 1.44 a
	80	58.36 ± 1.03 a	54.31 ± 1.14 b	62.31 ± 1.40 c	59.13 ± 1.05 a
Leaf	40	74.16 ± 1.78 a	88.32 ± 2.63 b	79.30 ± 1.66 c	77.38 ± 1.43 d
	80	72.64 ± 1.23 a	76.51 ± 1.33 b	77.51 ± 1.71 b	74.62 ± 1.77 c

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 $P \leq 0.05$ (DMRT).

Table 2 Effect of salt stress with or without TDM on amino acids content of different parts of *Vigna mungo* plants on different sampling days. Values are expressed in mg g-1 dry weight.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	176.6 ± 6.08 a	189.2 ± 6.51 b	182.3 ± 6.22 c	182.5 ± 6.23 c
	80	169.2 ± 5.51 a	182.6 ± 6.20 b	174.2 ± 6.52 c	175.1 ± 6.54 c
Stem	40	165.1 ± 5.41 a	178.9 ± 6.14 b	171.4 ± 6.48 c	171.5 ± 5.90 c
	80	158.4 ± 4.34 a	171.3 ± 5.95 b	163.8 ± 5.22 c	164.6 ± 5.40 c
Leaf	40	191.4 ± 6.52 a	204.5 ± 8.36 b	197.2 ± 6.87 c	197.2 ± 6.88 c
	80	184.3 ± 6.31 a	197.9 ± 6.88 b	189.9 ± 6.56 c	190.8 ± 6.53 d

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 $P \leq 0.05$ (DMRT).

Table 3 Effect of salt stress with or without TDM on proline content of different parts of *Vigna mungo* plants on different sampling days.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	169.1 ± 5.81 a	182.5 ± 6.88 b	175.3 ± 6.16 c	173.3 ± 6.03 c
	80	162.6 ± 5.31 a	176.0 ± 6.38 b	167.2 ± 5.56 c	166.6 ± 5.52 c
Stem	40	158.3 ± 5.22 a	171.6 ± 6.34 b	164.6 ± 5.51 c	158.2 ± 5.41 a
	80	151.6 ± 5.30 a	162.5 ± 6.58 b	156.2 ± 4.56 c	151.5 ± 4.93 a
Leaf	40	184.7 ± 6.72 a	187.1 ± 6.94 b	190.9 ± 7.02 c	147.3 ± 4.53 d
	80	177.4 ± 6.33 a	189.7 ± 6.87 b	182.5 ± 6.24 c	140.8 ± 4.03 d

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 $P \leq 0.05$ (DMRT).

Table 4 Effect of salt stress with or without TDM on glycine betain content of different parts of *Vigna mungo* plants on different sampling days.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	146.6 ± 5.33 a	159.7 ± 5.48 b	152.6 ± 5.24 c	147.3 ± 4.03 a
	80	139.8 ± 3.37 a	152.1 ± 5.87 b	144.2 ± 4.22 c	154.1 ± 5.53 d
Stem	40	135.5 ± 3.43 a	148.1 ± 5.54 b	141.1 ± 4.64 c	142.6 ± 4.87 c
	80	128.2 ± 3.83 a	141.7 ± 5.82 b	133.4 ± 3.29 c	136.3 ± 3.93 d
Leaf	40	161.6 ± 6.33 a	174.6 ± 6.87 b	167.6 ± 6.23 c	166.3 ± 5.03 c
	80	154.7 ± 5.37 a	167.9 ± 6.07 b	159.5 ± 5.88 c	162.0 ± 5.08 d

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 $P \leq 0.05$ (DMRT).

Table 5 Effect of salt stress with or without TDM on APX activity of different parts of *Vigna mungo* plants on different sampling days.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	161.2 ± 4.87 a	172.3 ± 4.78 b	178.3 ± 4.96 c	186.6 ± 4.99 d
	80	158.6 ± 4.45 a	165.2 ± 4.70 b	170.2 ± 4.89 c	179.8 ± 4.78 d
Stem	40	144.1 ± 4.39 a	156.0 ± 4.44 b	162.4 ± 4.88 c	169.1 ± 4.61 d
	80	141.0 ± 4.28 a	149.4 ± 4.42 b	154.1 ± 4.65 c	162.7 ± 4.52 d
Leaf	40	168.5 ± 4.68 a	181.7 ± 5.09 b	187.1 ± 4.99 c	194.8 ± 5.59 d
	80	164.4 ± 4.69 a	174.6 ± 4.98 b	179.3 ± 4.98 c	187.7 ± 5.01 d

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 $P \leq 0.05$ (DMRT).

Table 6 Effect of salt stress with or without TDM on CAT activity of different parts of *Vigna mungo* plants on different sampling days.

Plant parts	Sampling days	Control	100 mM NaCl	100 mM NaCl + 20 mg/L TDM	20 mg/L TDM
Root	40	151.2 ± 4.59 a	159.4 ± 4.61 b	165.1 ± 4.78 c	174.6 ± 4.98 d
	80	147.0 ± 3.26 a	152.4 ± 4.23 b	157.7 ± 4.31 c	165.7 ± 4.89 d
Stem	40	137.7 ± 3.02 a	143.7 ± 4.22 b	149.6 ± 4.12 c	157.1 ± 4.25 d
	80	134.9 ± 3.12 a	136.5 ± 3.57 a	141.1 ± 4.04 b	150.9 ± 4.09 c
Leaf	40	124.2 ± 3.01 a	129.9 ± 2.59 b	135.3 ± 3.50 c	142.5 ± 3.04 d
	80	119.4 ± 2.89 a	122.9 ± 2.23 b	127.1 ± 2.24 c	135.6 ± 3.03 d

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 $P \leq 0.05$ (DMRT).

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.* 2002). Application of TDM increased APX activity in salt-stressed plants 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).

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 H_2O_2 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.

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