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ENHANCEMENT OF ANTIOXIDANT ENZYME ACTIVITIES AND PRIMARY PHOTOCHEMICAL REACTIONS IN RESPONSE TO FOLIAR APPLICATION OF THIOLS IN WATER-STRESSED PEARL MILLET

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Primary photochemical reactions and the activities of the antioxidant enzymes chloroplastic superoxide dismutase (SOD), glutathione reductase (GR) and glutathione-S-transferase (GST) were determined in water-stressed pearl millet (*Pennisetum glaucum* L. cv. HHB-67) plants sprayed with the thiol compounds dithiothreitol (DTT), thioglycolic acid (TGA) and thiourea (TU) and the thiol modifiers 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and N-ethylmaleimide (NEM) at the earhead emergence stage (47 days after sowing, DAS), together with a control. Sampling was done at 54 and 67 days after sowing. Photosystem I and II (PS I and II) activities (ferricyanide site) were found to increase in plants sprayed with TU, TGA and DTT at both stages (54 and 67 DAS), but a reduction in PS II activity (DCQ Site) compared with the control was caused by NEM (66.66%) and DTNB (27.77%) at 54 DAS. A similar decrease in the activity of PS II (ferricyanide site) was found at 67 DAS for DTNB (55.55%). The chloroplastic SOD activity increased in chloroplasts isolated from leaves sprayed with thiol compounds at both sampling stages, except for NEM at 54 and 67 DAS. The activities of GR and GST in the leaves were higher in thiol-treated plants than in the control at 54 and 67 DAS, while the lowest GR activity was seen for the sulphydryl modifiers (DTNB and NEM) in leaves at 54 DAS. The experimental data suggest an enhancement in the primary photochemistry and antioxidant enzyme activities of water-stressed pearl millet in response to foliar spraying with thiol compounds.

Key words: antioxidant enzymes, foliar spray, photochemistry, sulphydryl compounds, thiols

Abbreviations: $^{\cdot}O^{-}_{2}$, singlet oxygen; CAT, catalase; APOX, ascorbate peroxidase; DAS, days after sowing; DCQ, 2,6-dichloro-p-benzoquinone; DTNB, 5,5'-dithio-bis-(2-nitrobenzoic) acid; DTT, dithiothreitol; EDTA, ethylene diamine tetra acetic acid; GPOX, guaiacol peroxidase; GR, glutathione reductase; GSH, glutathione (reduced); GSSG, glutathione (oxidised); GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; NBT, nitroblue tetrazolium; NEM, N-ethylmaleimide; PCMB**,** p-chloro-mercuric benzoate; OH^{-}, hydroxyl radical; PS I and II, Photosystem I and II; PVP, polyvinyl pyrrolidone; ROS, reactive oxygen species; SOD, superoxide dismutase; TGA, thioglycolic acid; TU, thiourea.

Introduction

In plants exposed to drought and high temperature stress a variety of reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^{\cdot}O^-_2$), are formed, leading to oxidative damage (Foyer et al., 1994). The efficient detoxification of O_2^- and H_2O_2 requires the coordinated action of several antioxidant enzymes, such as catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APOX; EC 1.11.1.11), guaiacol peroxidase (GPOX; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1), glutathione reductase (GR; EC 1.6.4.2) and glutathione-S-transferase (GST; EC 2.5.1.18). The superoxide radical is converted to H_2O_2 by the action of SOD, whereas CAT converts H_2O_2 to H_2O and molecular O_2. H_2O_2 is also detoxified by peroxidases with the help of a thiol, glutathione, as reductant (Noctor and Foyer, 1998), which plays an important role as an antioxidant in cells. Tolerant genotypes of tomato and maize accumulated more GSH during chilling and had constitutively higher GR activity than sensitive ones (Walker and McKersie, 1993; Kocsy et al., 1996; 1997), corroborating the involvement of GSH and GR in increased stress tolerance. Lipid peroxidation by-products such as organic peroxides are eliminated from the cell with the help of glutathione peroxidase and GR. The non-enzymatic antioxidant defence system includes reductants such as ascorbate and glutathione. Besides antioxidant protection, the intracellular and extracellular redox states of thiols play a critical role in the stabilization of protein structure and function, the regulation of enzyme activity and the control of transcription factor activity (Deneke, 2000; Sen, 2000; Ramaswamy et al., 2007). Sulphydryl compounds such as dithiothreitol and glutathione also enhance stomatal opening in epidermal strips both in light and darkness, while the sulphydryl reagent/sulphydryl modifier N-ethyl maleimide (NEM) inhibits stomatal opening, indicating the possible involvement of sulphydryl groups in stomatal movements (Madhusudana and Anderson, 1983). Dithiothreitol (DTT) stimulated enzyme activity in the leaves of *Trillium apetalon*, but it was strongly inhibited by sulphydryl group modifiers (PCMB and iodoacetate). The sulphydryl group modifiers (PCMB and NEM) reduced photosynthetic efflux and their inhibitory effect was reversed by DTT (Fieuw and Patrick, 1993). It has been reported that compounds such as mercaptoethanol, mercaptoethylamine and thiourea improve the productivity of maize (Sahu and Solanki, 1991; Sahu et al., 1993).

Apart from playing a role in productivity these thiol compounds may also be involved in the antioxidant defence response of the plant. Sulphydryl compounds can prevent the denaturation of membrane proteins and thus help to maintain the plasma-membrane integrity under drought stress. Better yields may be obtained even under adverse environmental conditions if the plant is able to generate increased reductants via a stimulative response to oxidative stress. This paper reports the enhancement of the primary photochemistry and antioxidant defence response after foliar spraying with thiols, thus improving the stress tolerance of pearl millet.

Materials and methods

Plants, growth conditions and experimental treatments

Pearl millet plants (*Pennisetum glaucum* cv. HHB-67) were grown in the Green House Laboratory at BARC, Mumbai, in earthen pots, each filled with 5 kg of soil. Before sowing, the pots were supplied with a balanced dose of NPKS fertilizers @ 90:40:60:40 kg ha^{-1} on a dry weight basis of one furrow slice (i.e. 2.24 x 10^6/kg ha^{-1} up to 15 cm depth). Half the of nitrogen and the full dose of PKS were given at the time of pot filling and the remaining nitrogen 30 days after sowing (DAS). After fertilizer application the pots were saturated with water and allowed to settle over night. The seeds were disinfected with 70% alcohol for 2 min before sowing. Forty-two days after sowing, at the pre-flowering stage, the plants were subjected to water stress for five days. Thereafter, irrigation was provided on alternate days, when the plants usually showed symptoms of incipient wilting. This intermittent mild water stress was continued till harvest. The earhead fully emerged at 54 DAS and grain formation started at 67 DAS. The sulphydryl compounds DTT (0.07 mM), TGA (1.4 mM), TU (6.6 mM), DTNB (0.13 mM), DTNB + TU (0.13 mM + 6.6 mM), NEM (0.8 mM) and NEM + TU (0.8 mM + 6.6 mM) were sprayed at earhead emergence (47 DAS). Leaves were excised from each treatment at 54 DAS (8 days after foliar spraying) and 67 DAS (20 days after spraying) for the isolation of chloroplasts and enzymes. All the chemicals used for the experiments were procured from the Sigma Chemical Co.

Chloroplast isolation, photosystem (PS) I and II activities

Chloroplasts were isolated from pearl millet leaves following the method of Izawa and Good (1968). PS I activity was measured polarographically in a Clark-type O_2 electrode (Gilson, Saint Louis, USA) at 21°C in rate-saturating red light as methyl viologen (MeV)-mediated O_2 uptake by a chloroplast suspension equivalent to 20 µg chlorophyll (Chl) cm^{-3}, as described by Izawa (1980). PS II activity was measured as oxygen evolution by chloroplasts [20 µg(Chl) cm^{-3}] using either 0.3 mM 2,6-dichloro-*p*-benzoquinone (DCQ) or 0.4 mM potassium ferricyanide [$K_3Fe(CN)_6$] as electron acceptors, as described by Nayak et al. (2003).

Enzyme extraction and assay

Leaf samples (0.5 g fresh weight) were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone. The homogenate was filtered through four layers of cheesecloth and then centrifuged at 4°C for 20 min at 15,000 g. The supernatant was collected and an appropriate aliquot/dilution of the crude extract was used for antioxidant enzyme (GR and GST) assays. All parts of the enzyme extraction were performed at 0–4°C and the enzyme assays were carried out at room temperature (23 ± 1°C), unless otherwise stated.

The chloroplastic-SOD activity was estimated in isolated chloroplasts by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), as detailed by Becana et al. (1986). The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 14.3 mM methionine, 82.5 µM nitroblue tetrazolium and 2.2 µM riboflavin. The system was placed 30 cm below the source of irradiance (1400 µmol photons m^{-2} s^{-1}). The reaction was run for 30 min and stopped by switching the lights off. The reduction of nitroblue tetrazolium was monitored by reading the absorbance at 560 nm. One unit of SOD (U) was defined as the amount of enzyme that produced 50% inhibition of nitroblue tetrazolium reduction under the assay conditions, as described by Giannopolitis and Ries (1977).

GR activity was measured by monitoring the decrease in absorbance at 340 nm (ε = 6.2 mM^{-1} cm^{-1}) for 1 min. The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 0.5 mM GSSG, 0.1 mM EDTA, 3 mM $MgCl_2$ and 0.15 mM NADPH, as described by Shaedle and Bassham (1977). Activity was expressed as µmol NADPH oxidised mg^{-1} protein min^{-1}.

GST activity was measured as per Mannervik and Guthenberg (1981) by following changes in the absorbance at 340 nm for 1 min in a mixture containing 100 mM sodium phosphate buffer (pH 6.5), 1 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene. The activity of GST was expressed as µmol 2,4-dinitrophenyl glutathione formed mg^{-1} protein min^{-1} (ε = 9.8 mM^{-1} cm^{-1}).

Statistical analysis of data

The data were statistically evaluated using a factorial completely randomized design (CRD) with three replicates (Raghavarao, 1983).

Results

PS II activity

Table 1 shows that in water-stressed plants, PS II activity was increased by foliar sprays of –SH compounds by nearly 75% (TU and TGA) and 37.50% (DTT) in comparison with the respective unsprayed control at 54 DAS, when $K_3Fe(CN)_6$ was used as an electron acceptor. When DCQ was used as electron acceptor, the PS II activity was significantly increased by DTT (30.0%) and TGA (27.77%) at 54 DAS as compared to the control. However, an inhibitory effect on PS II activity was observed for NEM (66.66%) and DTNB (27.77%) compared to the unsprayed control. This inhibitory effect was significantly reversed when TU spray was applied 24 h after DTNB and NEM. Likewise, at 67 DAS, there was a 33.33% increase in PS II activity (ferricyanide site) with foliar sprays of TU, TGA and DTT, compared to the control, while a 55.55% decline in PS II activity was observed with DTNB. It can be seen that spraying with TU significantly improved the PS II activity of DTNB-treated plants at 67 DAS, but the activity was still less than in unsprayed plants (Table 1).

PS I activity

Table 2 shows the PS I activity in chloroplasts isolated from pearl millet leaves after different treatments. Foliar spraying with thiol compounds (TU, TGA and DTT) significantly increased the PS I activity as compared to unsprayed plants. The sulphydryl modifiers (DTNB and NEM) also showed a stimulatory affect on PS I activity at both sampling dates. At 54 DAS, increased PS I activity was observed after foliar spraying with TU (52.83%) and TGA (39.62%) compared with control plants. At 67 DAS, the highest increase in PS I activity was found for TU (24%) followed by TGA (21.33%) and DTT (21.33%) as compared with control plants. Though DTNB and NEM are sulphydryl blocker reagents, they did not inhibit PS I activity except for NEM at 67 DAS (Table 2).

Table 1

Effect of foliar spraying on PS II activities (μmol O_2 evolved mg^{-1} Chl h^{-1}) in chloroplasts isolated from pearl millet. The data represent the means of three independent sets of experiments

Treatments	$H_2O \rightarrow K_3Fe(CN)_6$		$H_2O \rightarrow DCQ$
	54 DAS	67 DAS	54 DAS
Control	120	135	90
DTT (0.07 mM)	165	180	117
TGA (1.4 mM)	210	180	115
TU (6.6 mM)	210	180	95
DTNB (0.13 mM)	150	60	65
DTNB + TU (0.13 + 6.6 mM)	165	115	113
NEM (0.8 mM)	150	135	30
NEM + TU (0.8 + 6.6 mM)	152	135	65
C.D. at 5%	36.86	30.42	18.96

DAS: Days after sowing

Table 2
Effect of foliar spraying on PS I (MV → H_2O) activities (μmol O_2 consumed mg^{-1} Chl h^{-1}) in chloroplasts isolated from pearl millet. The data represent the means of three independent sets of experiments

Treatments	54 DAS	67 DAS
Control	106	150
DTT (0.07 mM)	135	182
TGA (1.4 mM)	148	182
TU (6.6 mM)	162	186
DTNB (0.13 mM)	162	185
DTNB + TU (0.13 + 6.6 mM)	150	186
NEM (0.8 mM)	136	140
NEM + TU (0.8 + 6.6 mM)	136	150
C.D. at 5%	32.46	28.23

DAS: Days after sowing

Chloroplastic SOD activity

At 54 DAS the activity of chloroplastic-SOD in the leaves (Fig. 1) increased significantly to the highest level in plants sprayed with TGA (34.27%), TU (19.72%) and DTT (19.31%) compared with unsprayed control plants. However, NEM significantly inhibited the chloroplastic SOD activity (9.53%). When the plants were sprayed with NEM followed by TU (24 h later), the inhibitory effect of NEM on enzyme activity was significantly reversed by TU. At 67 DAS foliar spraying with DTT resulted in a significant increase (18.15%) in chloroplastic SOD activity in the leaves as compared to the untreated control. In contrast the chloroplastic SOD activity declined after foliar spraying with sulphydryl group modifiers, by 19.20% for DTNB and 16.23% for NEM. However, the inhibitory effect of DTNB and NEM was significantly reversed by TU (Fig. 1).

GR activity

At 54 DAS the GR activity in the leaves increased in plants sprayed with TGA (67.42%), TU (34.38%) and DTT (27.19%) in comparison with the control (Fig. 2), while low GR activities in leaves were observed with sulphydryl group modifiers (DTNB and NEM). The reduction was significant (17.08%) for DTNB, but marginal for NEM. However, when NEM was followed by TU (after 24 h) the effect on enzyme activity was reversed (16.05%) compared with NEM alone. However, foliar spraying with TU in DTNB-treated plants did not have any reversing effect. At 67 DAS the respective increase in GR activity in the leaves was 65.15%, 48.77%, 39.36%, 26.46%, 15.66% and 13.58% for TU, DTNB + TU, DTT, NEM + TU, DTNB and NEM over the unsprayed control. No inhibitory effect on the GR activity in the leaves was observed when the plants were sprayed with sulphydryl group modifiers (DTNB and NEM), and at 67 DAS higher GR activity was observed when DTNB and NEM were followed 24 h later by TU than in the control (Fig. 2).

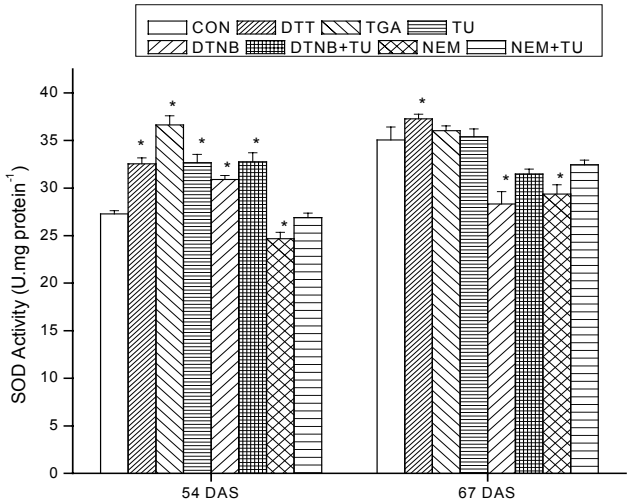

Fig. 1. Effect of foliar spraying with 0.07 mM DTT, 1.4 mM TGA, 6.6 mM TU, 0.13 mM DTNB, (0.13 + 6.6) mM DTNB + TU, 0.8 mM NEM or (0.8 + 6.6 mM) NEM + TU on the chloroplastic SOD activity in pearl millet. The data represent the means ± S.E. of three independent sets of experiments and * expresses significant changes in enzyme activities. CON: Control, DAS: Days after sowing

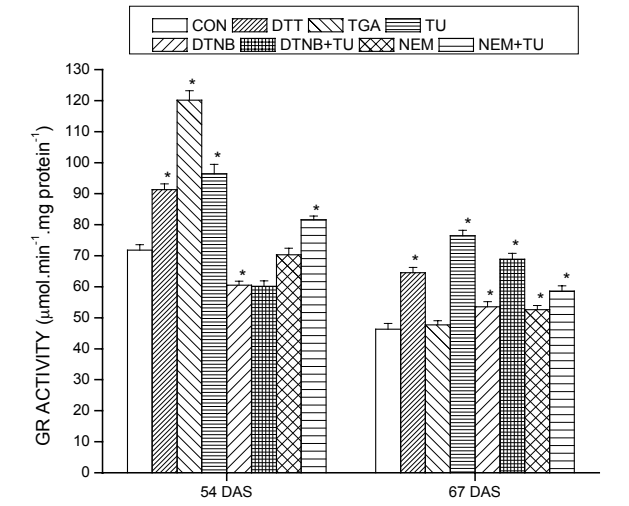

Fig. 2. Effect of foliar spraying with 0.07 mM DTT, 1.4 mM TGA, 6.6 mM TU, 0.13 mM DTNB, (0.13 + 6.6) mM DTNB + TU, 0.8 mM NEM or (0.8 + 6.6 mM) NEM + TU on the GR activity in the leaves of pearl millet. The data represent the means ± S.E. of three independent sets of experiments and * expresses significant changes in enzyme activities. CON: Control, DAS: Days after sowing

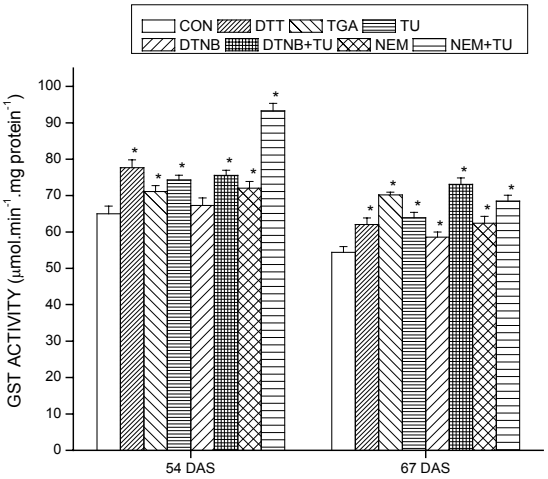

Fig. 3. Effect of foliar spraying with 0.07 mM DTT, 1.4 mM TGA, 6.6 mM TU, 0.13 mM DTNB, (0.13 + 6.6) mM DTNB + TU, 0.8 mM NEM or (0.8 + 6.6 mM) NEM + TU on the GST activity in the leaves of pearl millet. The data represent the means ± S.E. of three independent sets of experiments and * expresses significant changes in enzyme activities. CON: Control, DAS: Days after sowing

GST activity

Thiol treatments increased the GST activity in the leaves at both sampling dates (Fig. 3). At 54 DAS the increases after foliar spraying with DTT, TU and TGA were 19.46%, 14.27% and 9.41%, respectively, in comparison to the unsprayed control. The inhibitory effect of DTNB and NEM was not seen in respect of GST activity, which was higher in the NEM + TU (43.47%) and DTNB + TU (16.16%) treatments compared to the unsprayed control (Fig. 3). At 67 DAS the highest GST activity in the leaves was recorded for TGA (29.09%), followed by TU (17.45%) and DTT (14.08%), respectively, over the control. GST activity was not inhibited by sulphydryl group modifiers (DTNB and NEM) at either sampling date, so the values were higher after spraying with DTNB + TU (34.35%) or NEM + TU (25.89%) than in the unsprayed control (Fig. 3).

Discussion

It is known that drought stress induces physiological, biochemical and molecular responses in crop plants, helping them to adapt to the limiting environmental conditions (Arora et al., 2002). Photosynthesis decreases under water deficit conditions, resulting in the inhibition of electron transfer, which in turn leads to the formation of ROS that can initiate photo-oxidative damage (Asada, 1999). Thiols are thought to play a pivotal role in protecting cells

against oxidative stress. From Tables 1 and 2 it is clear that thiol treatment with TU, TGA and DTT increased the PS II and PS I activity at both sampling dates when ferricyanide/DCQ was used as the electron acceptor. However, a significant decrease in PSII activity (DCQ) was observed when sulphydryl group modifiers (DTNB, NEM) were sprayed on the plants. This inhibitory effect of DTNB and NEM was reversed by foliar spraying with TU at 54 DAS (Table 1). It should be noted that DTNB is a plasma membrane non-permeant –SH reagent, whereas NEM is a membrane permeant –SH reagent. In other words, DTNB acts on membrane-bound proteins and their –SH groups, whereas NEM can enter the cytosol and act on cytoplasmic proteins. Studies on wheat subjected to water stress indicated that the PS I and II activities were less affected by seed soaking with sulphydryl compounds (Nathawat et al., 2007). It has also been reported that foliar spraying with TU increased both canopy photosynthesis and the photosynthetically active leaf area in maize (Sahu et al., 1993). DTT has also been reported to stimulate CO_2 assimilation in the dark (Werdan et al., 1975). The redox states of thiols are involved in regulating the activity of enzymes and transcription factors, so foliar spraying with thiols may have resulted in an up-regulation of the plant antioxidant defence. In the present investigation foliar spraying with thiols (DTT, TGA and TU) significantly increased the chloroplastic SOD activity, but NEM had an inhibitory effect. In NEM-sprayed plants, TU improved the chloroplastic SOD activity at both sampling stages (Fig. 1). An increase in SOD activity was observed in pearl millet seeds pre-soaked with sulphydryl compounds during water stress (Ramaswamy et al., 2007). The over-expression of SOD was found to act as a safeguard against drought (McKersie et al., 1996) and salinity (Hasegawa et al., 2000; Zhu et al., 2002).

When biological systems dehydrate, the resulting loss in enzyme activity, such as glutathione reductase and NADPH-generating pathways, leads to an increase in the oxidative environment. In the present experiment, high thiol-mediated antioxidant enzyme activities (GR and GST) were observed in the leaves after foliar spraying with sulphydryl compounds (DTT, TGA, TU) at both sampling stages (Figs. 2 and 3). However, decreased GR activities were noted with sulphydryl reagents (DTNB and NEM) over the unsprayed control. This inhibitory effect of DTNB and NEM on enzyme activities was reversed by foliar spraying with TU at 54 DAS (Fig. 2). High GR activity increases the $NADP^+$/NADPH ratio, thereby ensuring a continuous supply of $NADP^+$ to accept electrons from the photosynthetic electron transport chain and regenerating ascorbate in the process (Noctor et al., 2002). The significantly high antioxidant enzyme activities in the thiol treatments clearly demonstrate the greater efficiency of the antioxidant system in thiol-sprayed plants. Plants have evolved cellular adaptive responses, like upregulation of the antioxidant defence system and antioxidant stress protectors (Horling et al., 2003). Tolerant genotypes of tomato and maize accumulated more GSH during chilling and had constitutively higher GR activity than sensitive ones (Walker and McKersie, 1993; Kocsy et al., 1996; 1997), corroborating the involvement of GSH and GR in increased stress tolerance.

There are few reports on the effect of thiols on the antioxidant system. In similar studies, it was shown that treatment with dimethyl thiourea was able to trap H_2O_2 and also decrease the expression of APOX in maize roots (De Zacchini and De Agazio, 2001). Dimethyl thiourea has been shown to specifically scavenge hydroxyl radicals, whereas thiourea has been widely used to study the role of hydroxyl radicals in metal-mediated biological damage both *in vitro* and *in vivo*, though most of the data available are from animal studies (Zhu et al., 2002). The long-lasting effects of foliar applied thiols may result mostly from the amplification and regulation of signal transduction pathways under drought stress. This assumption is supported by the observation of Jia and Zhang (2000) that disulphide bonds or sulphydryl groups are critical to the reactivity of signal element(s) in the signalling process under water stress and that the sulphydryl group on the cellular domain is involved in the activation of a speculated water stress receptor protein located on the plasmalemma.

The results presented here show the enhancement of the antioxidant defence system and an increase in primary photochemical reactions under drought stress in pearl millet following foliar spraying with bioactive thiols. It is still not clear how these thiols regulate the antioxidant defence mechanism at the cellular level (signal transduction pathways that mediate plant responses to water stress are poorly understood), though the activities of the antioxidant enzymes are clearly elevated. The entrapment of ROS by thiols may play an important role in this process. A better understanding of the biochemical aspects of the water stress tolerance mechanisms imparted by foliar spraying with thiols could help to increase the productivity of pearl millet in a water-limited environment.

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References

Arora, A., Sairam, R. K., Srivastava, G. C. (2002): Oxidative stress and antioxidative systems in plants. *Curr. Sci*., **82**, 1227–1238.

Asada, K. (1999): The water-water cycle in chloroplast: Scavenging of active oxygen and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **50**, 601–639.

Becana, M., Aparicio-Tejo, P., Irigoyen, J. J., Sanchez-Diaz, M. (1986): Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*. *Plant Physiol*., **82**, 1169–1171.

De Zacchini, M., De Agazio, M. (2001): Dimethylthiourea, a hydrogen peroxide trap, partially prevents stress effects and ascorbate peroxidase increase in spermidine-treated maize roots. *Plant Cell Environ*., **24**, 237–244.

Deneke, S. M. (2000): Thiol-based antioxidants. *Curr. Top. Cell Regul*., **36**, 151–180.

Fieuw, S., Patrick, J. W. (1993): Mechanism of photosynthate efflux from *Vicia faba* L. seed coats. *J. Exp. Bot*., **44**, 65–74.

Foyer, C. H., Lelandais, M., Kunert, K. J. (1994): Photooxidative stress in plants. *Physiol. Plant.,* **92**, 696–717.

Giannopolitis, C. N., Ries, S. K. (1977): Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol*., **59**, 309–314.

Hasegawa, P. M., Ray, A. B., Kang, Z., Hans, J. B. (2000): Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol*. *Plant Mol. Biol*., **51**, 463–491.

Horling, F., Lamkemeyer, P., Konnig, J., Finkemeir, L., Kandlbinder, A., Baier, M., Dietz, K. I. (2003): Divergent light, ascorbate, and oxidative stress-dependent regulation expression of the peroxyredoxin gene family in *Arabidopsis*. *Plant Physiol.*, **131**, 317–325.

Izawa, S. (1980): Acceptors and donors for chloroplast electron transport. *Methods Enzymol*., **69**, 413–434.

Izawa, S., Good, N. E. (1968): The stoichiometric relation of phosphorylation to electron transport in isolated chloroplasts. *Biochim. Biophys. Acta*, **162**, 380–391.

Jia, W., Zhang, J. (2000): Water stress-induced abscisic acid accumulation in relation to reducing agents and sulphydryl modifiers in maize plants. *Plant Cell Environ*., **23**, 1389–1395.

Kocsy, G., Brunner, M., Ruegsegger, A., Stamp, P., Brunold, C. (1996): Glutathione synthesis in maize genotypes with different sensitivity to chilling. *Planta*, **198**, 365–370.

Kocsy, G., Owttrim, G., Brander, K., Brunold, C. (1997): Effect of chilling on the diurnal rhythm of enzymes involved in protection against oxidative stress in a chilling tolerant and a chilling sensitive maize genotype. *Physiol. Plant*., **99**, 249–254.

Madhusudana, R., Anderson, L. E. (1983): Light and stomatal metabolism. I. Possible involvement of light modulation of enzymes in stomatal movement. *Plant Physiol.*, **71**, 451–455.

Mannervik, B., Guthenberg, C. (1981): Glutathione transferase (Human placenta). *Methods Enyzmol*., **77**, 231–235.

McKersie, D. B., Stephen, R. B., Erni, H., Olivier, L. (1996): Water-deficit tolerance and field performance of transgenic alfalfa over expressing superoxide dismutase. *Plant Physiol*., **111**, 1177–1181.

Nathawat, N. S., Nair, J. S., Kumawat, S. M., Yadava, N. S., Singh, G., Ramaswamy, N. K., Sahu, M. P., D'Souza, S. F. (2007): Effect of seed soaking with thiols on the antioxidant enzymes and photosystem activities in wheat subjected to water stress. *Biol. Plant*., **51**, 93–97.

Nayak, L., Biswal, B., Ramaswamy, N. K., Iyer, R. K., Nair, J. S., et al. (2003): Ultraviolet-A induced changes in photosystem II of thylakoids: effects of senescence and high growth temperature. *J. Photochem. Photobiol*., **70 B**, 59–65.

Noctor, G., Foyer, C. H. (1998): Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **49**, 249–279.

Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L., Foyer, C. H. (2002): Drought and oxidative load in the leaves of C_3 plants: a predominant role of photorespiration. *Ann. Bot*., **89**, 841–850.

Raghavarao, D. (1983): *Designs of Experiments, Statistical Techniques in Agricultural and Biological Research*. New Delhi and Oxford and IBH Publisher Company, pp. 120–127.

Ramaswamy, N. K., Nathawat, N. S., Nair, J. S., Sharma, H. R., Kumawat, S. M., Singh, G., Sahu, M. P., D'Souza, S. F. (2007): Effect of seed soaking with sulphydryl compound on the photochemical efficiency and antioxidant defense system during the growth of pearl millet under water limiting environment. *Photosynthetica*, **45**, 477–480.

Sahu, M. P., Solanki, N. S. (1991): Role of sulphydryl compounds in improving dry matter partitioning and grain production of maize (*Zea mays* L.). *J. Agron. Crop Sci*., **167**, 356–359.

Sahu, M. P., Solanki, N. S., Dashora, L. N. (1993): Effects of thiourea, thiamine and ascorbic acid on growth and yield of maize (*Zea mays* L.). *J. Agron. Crop Sci*., **171**, 65–69.

Sen, C. K. (2000): Cellular thiols and redox-regulated signal transduction. *Curr. Top. Cell Regul*., **36**, 1–30.

Shaedle, M., Bassham, J. A. (1997): Chloroplast glutathione reductase. *Plant Physiol*., **59**, 1011–1012.

Walker, M. A., McKersie, B. D. (1993): Role of the ascorbate glutathione antioxidant system in chilling resistance of tomato. *J. Plant Physiol.,* **141,** 234–239.

Werdan, K., Heldt, H. W., Milovancev, M. (1975): The role of pH in the regulation of carbon fixation in the chloroplast stroma: studies on CO_2 fixation in the light and dark. *Biochem. Biohys. Acta*, **396**, 276–282.

Zhu, B. Z., Antholine, W. E., Frei, B. (2002): Thiourea protects against copper-induced oxidative damage by formation of a redox-inactive thiourea-copper complex. *Free Rad. Biol. Med.*, **32**, 1333–1338.

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