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BRIEF COMMUNICATION

Effect of seed soaking with sulphydryl compounds on the photochemical efficiency and antioxidant defence system during the growth of pearl millet under water limiting environment

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Abstract

Pearl millet (*Pennisetum glaucum* L. cv. HHB-67) seeds were pre-soaked in sulphydryl compounds (dithiothreitol, thioglycollic acid, thiourea, and cysteine). In plants at 59 and 67 d after sowing (DAS), activities of photosystem (PS) 2 (ferricyanide site) and PS1, both chloroplastic and total superoxide dismutase, glutathione reductase, and glutathione-S-transferase increased after all sulphydryl pre-treatments at both stages of plant development. Also dry matter of plant parts sampled at 55 DAS was higher after thiol-treatments in comparison with control.

Additional key words: antioxidant enzymes; drought tolerance; photosystems 1 and 2; seed soaking; thiols.

Pearl millet (*Pennisetum glaucum* L.) is a principal food cereal, cultivated in about 27 million hectares area of drought-prone arid and semi regions of Africa and the Indian subcontinent. The crop is highly adapted to drought and is mostly grown under rain-fed environment without supplemental irrigation. Drought stress due to inadequate rains at pre-flowering stage adversely affects canopy photosynthesis and reproductive development of the crop, which eventually results in low crop yields. A consequence of the drought-induced limitation of photosynthesis is the over-reduction of PS2 reaction centres (Demmig-Adams and Adams 1992) and in the increased production of reactive oxygen species in chloroplasts (Smirnoff 1995). To counteract the toxicity of active oxygen species, plants use a highly efficient antioxidant defence system, represented mainly by glutathione (GSH), which protects many cellular components and the thiol status of proteins against oxidative stress (Gilbert *et al.* 1990). However, when biological system undergoes dehydration, activity of the enzymes such as glutathione reductase (GR) may decrease, resulting in formation and accumulation of disulfides (GSSG) and mixed disulfides between GSH and other thiol-containing protein molecules (PSSG). Thus, the antioxidant defence system generally protects the PSH (protein free sulphydryl group) and GSH from desiccation-induced oxidative injury such as irreversible formation of intermolecular cross-links in proteins (Kranner and Grill 1996). An early response of plants to water limitation relies on a rapid and massive accumulation of stress hormone abscisic acid (ABA) (Wright and Hiron 1969). Recent studies on the biochemical mechanism and signalling process of water stress-induced ABA accumulation have indicated that sulphydryl compounds, viz. dithiothreitol (DTT) and cysteine (Cys), significantly inhibit ABA accumulation (Jia and Zhang 2000). Seed soaking with sulphydryl compounds, mercaptoethanol and thiourea, increases dry matter (DM) accumulation and grain yield in pearl millet (Sharma 1988, Parihar *et al*. 1997, 1998). Seed soaking with thiourea (TU) also improved biological yield

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Abbreviations: ABA – abscisic acid; Cys – cysteine; DAS – days after sowing; DM – dry matter; DTT – dithiothreitol; EDTA – ethylene-diamine tetraacetic acid; GR – glutathione reductase; GSH – glutathione (reduced); GSSG – glutathione (oxidised), GST – glutathione-S-transferase; H_2O_2 – hydrogen peroxide; Mev – methyl viologen; MOPS – 3-[H-morpholine]propane sulphoxylic acid; NPKS – nitrogen, phosphorus, potassium, and sulphur; PS – photosystem; ROI – reactive oxygen intermediates; ROS – reactive oxygen species; -SH – sulphydryl group; SOD – superoxide dismutase; TGA – thioglycollic acid; TU – thiourea.

of maize (Sahu *et al*. 1993).

In view of the above, we investigated the effects of pre-soaking of seeds with sulphydryl compounds on anti-oxidant glutathione defence system and growth of pearl millet (*Pennisetum glaucum* L. cv. HHB-67) plants in water limited environment. Plants were raised in earthen pots, each of which was filled with 5 kg of soil. Before seed sowing, pots were supplied with balanced dose of NPKS (nitrogen, phosphorus, potassium, and sulphur, 90 : 40 : 60 : 40 kg per ha) fertilizer. Half dose of N and full dose of PKS were given at the time of pot filling and remaining half dose of N was given 30 d after sowing (DAS). After the application of nutrients in the above ratios, the pots were saturated with water and allowed to set overnight. Seeds were disinfected with 70 % alcohol for 2 min before sowing. Prior to sowing, seeds were pre-treated with dithiothreitol (DTT, 0.07 mM), thioglycollic acid (TGA, 1.4 mM), thiourea (TU, 6.6 mM), or cysteine (Cys, 0.83 mM) for 6 h, air dried, and used for sowing in pots. The control seeds were not pre-soaked in water: dry seeds were used for sowing. Forty five days after sowing (DAS), when plants were at pre-flowering stage, irrigation was withheld for 5 d. Irrigation was provided on alternate days, when plants usually showed symptoms of incipient wilting. Exposure of plants to such an intermittent mild water stress continued till harvest. At 59 DAS, the ear-head fully emerged, whereas at 67 DAS the grain formation started. All the chemicals used for the experiments and listed under abbreviations were procured from *Sigma Chemical Co*.

Chloroplasts were isolated from fully-grown leaves of the 59 and 67 DAS plants according to Izawa and Good (1968). Harvested leaves were transferred to ice-cold beaker and cut into fine pieces. Finely cut leaves were ground in 3–5 volumes (m/v*)* of grinding medium (30 mM Tricine, pH 7.8 containing 1 mM EDTA, 300 mM NaCl). The extract was filtered through 4 layers of cheesecloth and centrifuged at 4 340×*g* for 10 min. The pellet was washed with washing medium (10 mM MOPS, pH 7.4 containing 200 mM sucrose, 20 mM NaCl) by gently disturbing it with a brush so as to avoid clumps. The washed pellet was centrifuged at 121×*g* for 1 min. The supernatant was collected and re-centrifuged at 4 340×*g* for 10 min. The pellet was finally suspended in minimum volume of suspension medium (30 mM MOPS, pH 7.2 containing 10 mM NaCl).

PS1 activity was measured as methylviologen (MeV)-mediated O_2 uptake by chloroplast suspension equivalent to 20 g(Chl) m^{-3} as described by Izawa (1980). PS2-mediated oxygen evolution by chloroplasts equivalent to 20 g(Chl) m^{-3} was measured polarographically in a Clark-type O_2 electrode (*Gilson Scientific Instruments*, USA) at 21 °C in rate-saturating red radiation, using 0.4 mM potassium ferricyanide as electron acceptor, as described by Nayak *et al.* (2003).

Leaf samples (0.5 g fresh matter) were also homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1 % polyvinyl-pyrrolidone (PVP). The homogenate was filtered through four layers of cheesecloth and then centrifuged at 4 °C for 20 min at 27 000×*g*. The supernatant was collected and an appropriate aliquot/dilution of the crude extract of leaves was used for antioxidant enzyme [superoxide dismutase (SOD), glutathione reductase (GR), and glutathione-S-transferase (GST)] assays. All operations for the enzyme extraction were performed at 0–4 °C and the enzyme assays were carried out at room temperature (23±1 °C). The chloroplastic-SOD activity was estimated in isolated chloroplasts by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) as shown by Becana *et al.* (1986). The reaction mixture (3 cm^3) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 14.3 mM methionine, 82.5 μM NBT, and 2.2 μM riboflavin. The system was placed 30 cm from six 15 W fluorescent tubes. The reaction was run for 30 min and stopped by switching the light off. The reduction of NBT was followed by reading absorbance at 560 nm. One unit of SOD [U] was defined as the amount of enzyme that produced 50 % inhibition of NBT reduction as described by Giannopolitis and Ries (1977). Total SOD activity was estimated in a similar way in the enzyme extracts prepared from leaf samples. GR activity was measured according to Shaedle and Bassham (1977) by monitoring the decrease in absorbance at 340 nm for 1 min in the reaction mixture containing 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. GST activity was measured according to Mannervik and Guthenberg (1981), 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.

Chlorophyll (Chl) content was determined in 80 % acetone extracts of chloroplast suspension as described by Arnon (1949). Protein content in the leaf enzyme extracts was determined by the method of Bradford (1976), using bovine serum albumin as the standard.

Activities of PS2 and PS1 increased in chloroplasts isolated from leaves of plants raised from seeds soaked in DTT, TU, TGA, and Cys in the 59 and 67 DAS leaves (Table 1). However, in the 67 DAS leaves, PS2 activity showed only marginal increase caused by seed soaking in DTT, TU, and TGA solutions, as compared to control. In the 59 DAS leaves the activity of chloroplastic SOD (Fig. 1*A*) increased to highest level in plants grown from seeds soaked with Cys (33.7 %). The effects of TU and TGA over control were 30.6 and 14.0 %, respectively. In the 67 DAS leaves, the seed soaking with Cys and TGA also resulted in higher SOD activity in leaves as compared to the control (38.2 and 14.3 % increase, respectively). Thiol treatments increased total SOD activity (by 25.4 to 44.0 %) also in leaves at both sampling dates (Fig. 1*B*).

GR activity in the 59 DAS leaves was rather low (Fig. 1*C*). The percent increase in GR activity in leaves was highest with DTT (50.7 %) over control. However,

at 67 DAS the thiol pre-treatments markedly increased the enzyme activity, greatest difference being induced by TGA (1.45 fold) (Fig. 1*C*).

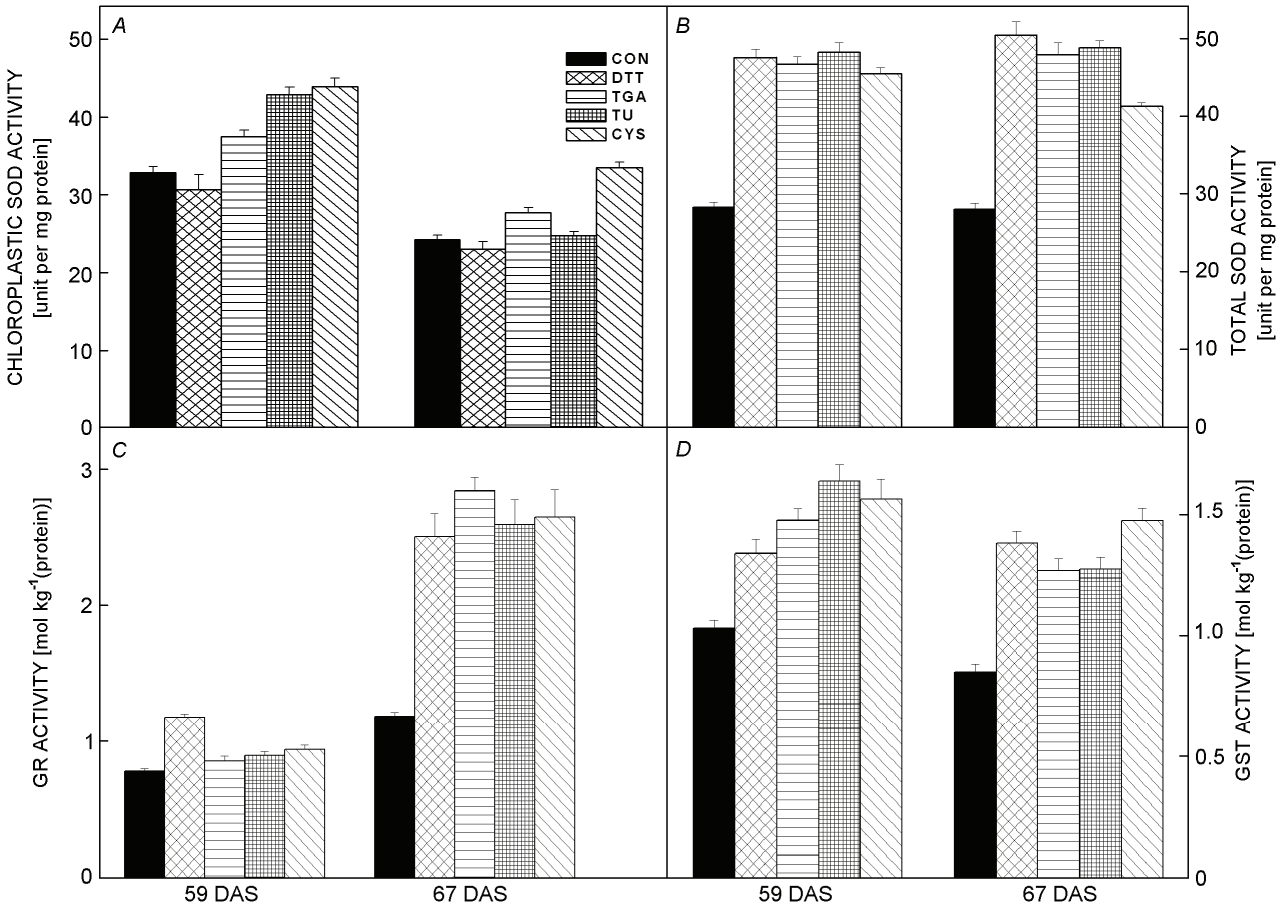

Fig. 1. Effects of seed pre-treatment with thiols on (*A*) chloroplastic and (*B*) total SOD activity, and (*C*) GR and (*D*) GST activities in leaves. Leaves were harvested 59 or 67 d after seed sowing. CON – control plants; treatments: dithiothreitol, DTT – 0.07 mM; thioglycollic acid, TGA – 1.40 mM; thiourea, TU – 6.60 mM; cysteine, Cys – 0.83 mM. Means ± S.E. of three independent sets of experiments.

Table 1. Photosystem (PS) 2 and 1 activities [μmol(O_2) kg^{-1}(Chl) s^{-1}] in chloroplasts isolated from pearl millet subjected to various thiol treatments (numbers in parentheses indicate percent increase or decrease over control), and dry matters, DM [g] of leaves, stem, and ear-head per plant at 55 DAS (numbers in parenthesis indicate actual DM of control).

Treatment	PS2		PS1		DM		
	59 DAS	67 DAS	59 DAS	67 DAS	Leaves	Stem	Ear-head
CON	33.3 ± 1.7	41.7 ± 2.5	41.7 ± 2.8	44.4 ± 2.2	100.00 (3.20)	100.00 (3.53)	100.00 (3.52)
DTT	37.5 ± 1.9 (13)	37.5 ± 2.8 (–10)	55.6 ± 3.1 (33)	48.6 ± 1.7 (9)	111.87	125.77	106.81
TGA	41.7 ± 2.5 (25)	54.2 ± 3.9 (30)	52.8 ± 3.6 (27)	45.2 ± 1.9 (2)	109.68	118.13	111.36
TU	45.8 ± 2.2 (38)	75.0 ± 4.7 (80)	55.6 ± 2.5 (33)	47.2 ± 2.5 (6)	105.31	110.48	115.05
Cys	37.5 ± 1.9 (13)	75.0 ± 5.6 (80)	48.6 ± 1.9 (17)	51.4 ± 2.2 (11)	109.68	120.67	131.25

GST activities in leaves were also higher in plants pre-treated with thiols (Fig. 1*D*). In the 59 DAS leaves, the highest GST activity in leaves was observed with TU (59.3 % increase). In the 67 DAS leaves, the increases in GST activity were up to 73.7 % (Cys).

DM of different plant parts (leaves, stem, and ear-head) determined at 55 DAS increased as a result of pre-treatments of seeds with thiols (Table 1). The percent increase in both stem and ear-head DM was highest with DTT (25.8 and 31.3 %, respectively).

In the 59 DAS leaves, the highest increase in PS2, PS1, and total SOD and GST activities was found after

TU pre-soaking (Table 1, Fig. 1*B,D*). Hence sulphydryl compounds protect by enhancing the photochemical efficiency, which has direct correlation with the antioxidant defence system in the pearl millet expressed at water limiting environment.

As a result, such thiol treated plants accumulated more dry matter in leaves, stem, and in reproductive structures (ear-heads) as compared to untreated control plants (Table 1). Similar results were observed in pearl millet by Sharma (1988) and Parihar *et al*. (1997, 1998). The result is that the treatment of seeds with thiol compounds improves tolerance of pearl plants to water stress.

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