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ENHANCING YIELD OF CLUSTERBEAN (*CYAMOPSIS TETRAGONOLOBA* L. TAUB) WITH FOLIAR APPLICATION OF SULPHYDRYL COMPOUNDS UNDER HOT ARID CONDITIONS

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SUMMARY

Water stress is one of the main environmental stress conditions that adversely affect growth and yield of crops. Sulphydryl (-SH) compounds have been reported to induce tolerance to abiotic stress conditions. A two-year (2010 and 2011) experiment was conducted at Bikaner, Rajasthan, India to test whether the exogenous applications of thiourea (500, 750 and 1000 mg L^{-1}) and thioglycolic acid (200, 300 and 400 mg L^{-1}) as a foliar spray would alleviate deleterious effects of water stress on clusterbean (*Cyamopsis tetragonoloba* L.). The -SH-treated plants showed higher membrane stability index, photosynthetic pigment content, photosynthetic capacity and antioxidant enzyme activities and lower lipid peroxidation compared with untreated plants. Compared with untreated plants, the -SH-treated plants had 11–18, 18–30, 17–57, 25–47, 14–22% higher membrane stability index, total chlorophyll content, antioxidant enzyme activities, net photosynthetic rate and seed yield, respectively; whereas the malondialdehyde content was 10–19% lower. These data suggest that under water deficit stress, exogenous -SH compound application improves photosynthesis by increasing photosynthetic pigment, protects plants against oxidative damage by scavenging reactive oxygen species and minimizing lipid peroxidation by elevated antioxidant enzyme activities. These results indicated the role of -SH compounds in diminishing the negative effects of water deficit on clusterbean and suggest that -SH compounds could be used as a potential bioregulator to improve plant growth and yield under water deficit conditions.

INTRODUCTION

Abiotic stress is a major cause of crop loss worldwide. Drought is one of the greatest abiotic stress conditions limiting crop productivity (Ashraf, 2010), particularly in arid and semi-arid regions of the world (Blum, 2005). Water stress adversely affects a variety of vital physiological and biochemical processes in plants such as reducing stomatal conductance, photosynthesis and enhancing pigment degradation (Hasanuzzaman *et al.*, 2012). Extensive studies on stress have demonstrated that exposure of plants to water stress induces overproduction of reactive oxygen species (ROS), such as the superoxide radical, hydrogen peroxide and hydroxyl radical in plant cells (Farooq *et al.*, 2009). ROS are highly reactive to membrane lipids, protein and DNA, causing oxidative damage and impairing normal functions of cells. These

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are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage. To minimize adverse effects of oxidative stress, plants have evolved complex enzymatic and non-enzymatic antioxidant systems such as low molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APOX)) (Apel and Hirt, 2004). Balance between ROS production and activities of antioxidant enzymes determines the occurrence of oxidative signalling and/or damage (Moller *et al.*, 2007). The enhancement of antioxidant defence mechanisms is considered to be an adaptive mechanism of plants to drought stress, and the strengthening of these defense mechanisms through the enhanced functions of antioxidant components may reduce or prevent oxidative damage and improve the drought resistance of plants (Hasanuzzaman *et al.*, 2012).

Exogenous application of stress alleviating agents is an important means to improve stress tolerance in field crops (Farooq *et al.*, 2009). The -SH containing compounds, such as thiourea (TU) and thioglycolic acid (TGA), are an important group of stress alleviating substances. Several studies support the major role of -SH compounds in modulating plant response to various abiotic stress conditions. These are reported to decrease denaturation of membrane protein (Rikin *et al.*, 1979), improve translocation of sucrose from source to sink via phloem loading (Srivastava *et al.*, 2008), enhance photosynthetic pigments (D'Souza *et al.*, 2009), net photosynthetic rate (Burman *et al.*, 2004) and N-metabolism (Garg *et al.*, 2006) and decrease stress-induced drop in photo-system I (PS I) and photo-system II (PS II) (Ramaswamy *et al.*, 2007) in plants under stress conditions.

Clusterbean (*Cyamopsis tetragonoloba* L. Taub) is an annual legume crop belonging to family Leguminosae. It is mainly cultivated in north-western India, Pakistan, Sudan and the southern USA (Texas and Oklahoma). It is a major source of galactomannans, which are used as thickening, gelling and suspending agents, viscosifier and emulsion stabilizers in paper, textile, paint, drilling, oil operation, civil engineering, agrochemistry, food, cosmetics and pharmaceutical industries (Gresta *et al.*, 2013). India is a major producer of clusterbean, and contributes to 80% of the world's total production of clusterbean (Pathak *et al.*, 2011), which is 0.75 to 1.0 million tonne annually. In India, Rajasthan alone contributes 70% of India's total production. The bulk of clusterbean production is generated from the north-western hot arid region. There the crops are frequently prone to water deficit stress due to low and erratic rainfall (Garg *et al.*, 2006).

To date, very little information is available regarding the effects of -SH compounds on antioxidant enzyme activities, lipid peroxidation, gas exchange parameters and yield of clusterbean under water limiting conditions. Therefore, the present field experiments were conducted with objectives to assess growth, yield, gas exchange parameters and antioxidant enzyme activities under moisture limiting conditions in clusterbean. This paper reports the results of two experiments that tested the hypothesis that exogenous application of -SH compounds could improve growth, yield, gas exchange parameters and activities of antioxidant enzymes of clusterbean under a water limiting environment.

Table 1. Weather data for the cropping period at Bikaner, Rajasthan, India.

	2010				2011			
Month	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Total rain-fall (mm)	Total evapora-tion (mm)	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Total rain-fall (mm)	Total evapora-tion (mm)
July	38.6	29.1	34.8	249.7	39.4	26.7	168.2	240.4
August	36.5	27.2	82.4	259.7	36.6	24.9	86.8	249.9
September	30.0	20.9	164.0	198.8	29.3	21.0	113.2	191.9
October	36.4	20.7	0.0	227.3	35.2	18.7	0.0	231.4
November	29.7	14.0	20.0	176.6	32.3	14.2	0.0	176.6

MATERIALS AND METHODS

Study environment

Field experiments were conducted during two kharif seasons (July–November) in 2010 and 2011 at the Central Arid Zone Research Institute, Regional Research Station, Bikaner (28°4′ N; 74°3′ E; 238.3 m above mean sea level), Rajasthan, India. The climate of the experimental site is hot arid with an average annual precipitation of 287 mm. More than 85% of the total annual rainfall is received during the south-west monsoon season (July to September). The weather data for the crop growing seasons during the two-year experiment are presented in Table 1. The soil was Torripssamentes typic with the following key properties for the 0–20-cm layer: pH (soil/H_2O, 1:2.5): 8.5, organic carbon: 1.5 g kg^{-1} (Walkley–Black), available P: 0.004 g kg^{-1} (Olsen) and available K: 0.107 g kg^{-1} (1 N NH_4-acetate). Texture was loamy sand, with sand (2000–50 μm), silt (50–2 μm) and clay (<2 μm) content of 864, 61 and 85 g kg^{-1} respectively.

Treatments and experimental design

The treatments included two -SH compounds and their concentrations, namely TGA (200, 300 and 400 mg L^{-1}) and TU (500, 750 and 1000 mg L^{-1}). An additional untreated control (water spray) was also included in the study. Thus, there were seven treatments. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Two foliar sprays of -SH compounds were applied at vegetative and pre-flowering stages of crop. Size of each plot was 5.0 × 3.5 m with 2-m gap in-between. Each plot was bordered with an earth dike of 30 cm in height. Overall, there were 21 plots for the whole experiment.

Crop management practices

The land was cleared manually with minimal soil disturbance in both years of the study. Prior to the pre-planting tillage operations during the growing seasons of the two years, thoroughly mixed farm yard manure (containing 0.33% N, 0.21% P and 0.39% K) was applied uniformly over the entire field at 2.5 M g manure ha^{-1}. After receiving adequate monsoon rain, the land was prepared by a tractor-drawn disc

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harrow. Clusterbean (cultivar RGC-1003) was sown on 1 August and 30 July in 2010 and 2011, respectively. In each plot, 20-cm deep furrows were created with the help of manual drawn plow and seeds were sown uniformly at ~10-cm depth. A basal dose of 10 kg N ha^{-1} (as urea) and 20 kg P ha^{-1} (as single superphosphate) was applied at sowing. Fluchloralin (45 EC) at a rate of 1 L ha^{-1} was applied before the sowing of the crop to control weeds.

Leaf sampling and determination of physio-biochemical traits

Leaf samples were taken at flowering stage (50 days after sowing (DAS)) from two plants per plot. Completely developed third and fourth leaves from the top of the plant were used for all measurements. Leaves were collected on ice between 0930–1030 h, and taken to the laboratory, washed with distilled water, and excess water was removed.

Determination of antioxidant enzyme activities

Leaf samples (0.5 g fresh weight) were homogenized in ice-cold 50-mM potassium phosphate buffer (pH 7.0) containing 0.1-mM ethylenediaminetetraaceatic acid (EDTA) and 1% (w/v) polyvinylpolypyrolidone. The homogenate was filtered through four layers of cheese cloth, and 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 enzyme assays. Complete operation of enzyme assays was carried out at room temperature (23 ± 1 °C) unless otherwise stated. The enzyme assay was performed for three replications.

The CAT activity was assessed following the method described by Chance and Maehly (1955). CAT activity was measured following the decomposition of H_2O_2 at 240 nm (coefficient of absorbance, $\varepsilon = 39.44\ mM^{-1}\ cm^{-1}$) in a reaction mixture (1 ml) contained 680 μL of 50-mM phosphate buffer (pH 7.0), 300 μL of 200-mM H_2O_2 and 20 μL of enzyme extract. CAT enzyme specific activity is expressed as μmol H_2O_2 decomposed mg^{-1} (protein) min^{-1}.

The SOD activity was determined by the method of Becana *et al.* (1986) based on the photochemical inhibition of *p*-nitro blue tetrazolium chloride (NBT). Reaction mixture (3.0 ml) contained 50-mM phosphate buffer (pH 7.8), 0.1-mM EDTA, 14.3-mM methionine, 82.5-μM NBT, 2.2-μM riboflavin and 50 μL of enzyme extract. Test tubes were irradiated under six 15-W florescent tubes for 30 min. The photo-reduction of NBT was measured at 560 nm with a UV/visible spectrophotometer. Blanks and controls were run in the same manner but without illumination and enzyme respectively. One unit of SOD activity was defined as extract volume that caused 50% inhibition of the photo-reduction of NBT.

The APOX activity was assayed by following decrease in absorbance at 290 due to ascorbate oxidation ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) in a reaction mixture (1 ml) contained 530 μL of 50-mM phosphate buffer (pH 7.0), 200 μL of 0.5-mM ascorbic acid, 200 μL of 0.1-mM H_2O_2, 50 μL of 0.1-mM EDTA and 20 μL of enzyme extract for 1 min following the method of Nakano and Asada (1981). APOX enzyme-specific activity is expressed as μmol ascorbate oxidized mg^{-1} (protein) min^{-1}.

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For guaiacol peroxidase (GPOX), the oxidation of guaiacol was measured by following the increase in absorbance at 470 nm ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) for 1 min. The assay mixture (1 ml) contained 530 μL of 50-mM phosphate buffer (pH 7.0), 50 μL of 0.1-mM EDTA, 200 μL of 10-mM guaiacol and 200 μL of 10-mM H_2O_2, and 20 μL of enzyme extract for 1 min as described by Chance and Maehly (1955). The GPOX activity is expressed as μmol (tetraguaiacol formed) mg^{-1} (protein) min^{-1}.

The glutathione reductase (GR) activity was measured as described by Shaedle and Bassham (1977) by following decrease in absorbance at 340 nm ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$) for 1 min. The reaction mixture (1 ml) contained 655 μL of 50-mM Tris-HCl buffer (pH 7.5), 125 μL of 0.5-mM oxidised glutathione (GSSG), 50 μL of 0.1-mM EDTA, 50 μL of 3-mM $MgCl_2$, 100 μL of 0.15-mM NADPH and 20 μL of enzyme extract. The GR activity was expressed as μmol (NADPH oxidized) mg^{-1} (protein) min^{-1}.

Determination of lipid peroxidation

The method of Cakmak and Horst (1991) was followed for the measurement of lipid peroxidation products in terms of 2-thio-barbituric acid reactive substances (TBARS), and expressed as equivalents of malondialdehyde (MDA). Fresh leaf sample (0.5 g) was ground in 10 ml of 10% tri-chloroaceatic acid (TCA) at 4 °C and centrifuged at 10,000 × g for 5 min. The 1-ml aliquot was taken and added to 4 ml of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA. It was then cooled in an ice bath and centrifuged at 10,000 × g for 5 min. The absorbance was then recorded at 532 and 600 nm. The MDA content was determined using the following formula:

$$\mathrm{MDA}(\mu\mathrm{molg}^{-1}\mathrm{FW}) = [(A532 - A600) \times V \times 1000/\varepsilon] \times W,$$

where ε is the specific extinction coefficient ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$), V is the 10-ml volume of grinding medium, W is the fresh weight of leaf, and $A600$ and $A532$ are the absorbance at 600- and 532-nm wavelengths respectively.

Determination of membrane stability index (MSI)

The MSI of leaf samples was determined following the procedure described by Sairam *et al.* (2002). Leaf samples (0.1 g) were cut into discs of uniform size and placed in 10 ml of double-distilled water in two sets. One set was kept at 40 °C for 30 min and its conductivity (C_1) was recorded. The second set was kept in boiling water bath (100 °C) for 10 min and its conductivity (C_2) was recorded. The conductivity was measured with a conductivity meter. MSI was calculated using the following formula: $\mathrm{MSI} = [1 - (C_1/C_2)] \times 100$.

Determination of photosynthetic pigments

Chlorophyll and carotenoid contents were extracted by the non-maceration method (Hiscox and Israelstam, 1979). Fresh leaves (0.05 g) were extracted in 10-ml dimethyl sulfoxide (DMSO) for 65 °C for 4 h. The amount of chlorophyll *a*, *b* and carotenoids was determined spectrophotometrically by reading the absorbance at 645, 663 and 470 nm respectively (Arnon, 1949).

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Determination of gas exchange parameters

Gas exchange parameters, i.e. net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured *in vivo* with a portable photosynthesis system (TPS-2 CO_2 Gas Analyzers, USA). Gas exchange measurements were made on fully expanded leaves located in the upper third part of the canopy. Three plants were selected randomly from the central 1 × 1-m area from each plot to measure gas exchange parameters. Measurements were done in sunny and clear weather 0900 and 1100 h. The instantaneous water use efficiency (WUE) was determined as P_N/E (Nogueira *et al.*, 2004).

Determination of growth, yield attributes and yield

Ten plants were randomly selected from the central 2 × 2-m area of each plot to measure leaf area (LA) and dry matter (DM) accumulation per plant at 50, 60 and 70 days after sowing. Leaf area index (LAI) was calculated as the ratio of leaf area to ground area. Yield components, i.e. number of pods plant^{-1} (NP), number of seeds pod^{-1} (NS) and 1000-seed weight (SW) were recorded for all the plants from the central 1 × 1-m area of each plot at harvest. At the harvest maturity stage of the crop (on 4 November in 2010, and 29 October in 2011), seed yield (SY) and total above-ground biomass (ABY) were determined on an area of 2 × 2 m from each plot by manual harvesting of plants 3 cm above the ground and allowed to dry in the field. DM of seed and straw was determined by drying sub-samples in a convection oven at 65 °C to a constant weight.

Statistical analysis

All measured parameters were tested for significant differences between treatments using analysis of variance (ANOVA) for a randomized complete block design. Wherever significant, separation of treatment means was achieved by the procedure of least significant difference (LSD) as described by Gomez and Gomez (1984); $p \leq 0.05$ was used as a critical limit for distinguishing the degree of variance between means. Pearson correlation coefficients were used to evaluate relationships between different parameters. The treatment × year interaction was not significant for any trait measured.

RESULTS

Climatic condition

The weather data during the cropping periods are presented in Table 1. The rainfall received after sowing of crops was 244 mm and 200 mm during 2010 and 2011 respectively. During both years, there was no rainfall for 45 days of sowing, and thus crops experienced prolonged terminal water deficit stress. The average monthly maximum temperatures from July to October were 34.3 °C and 33.7 °C for 2010 and 2011 respectively.

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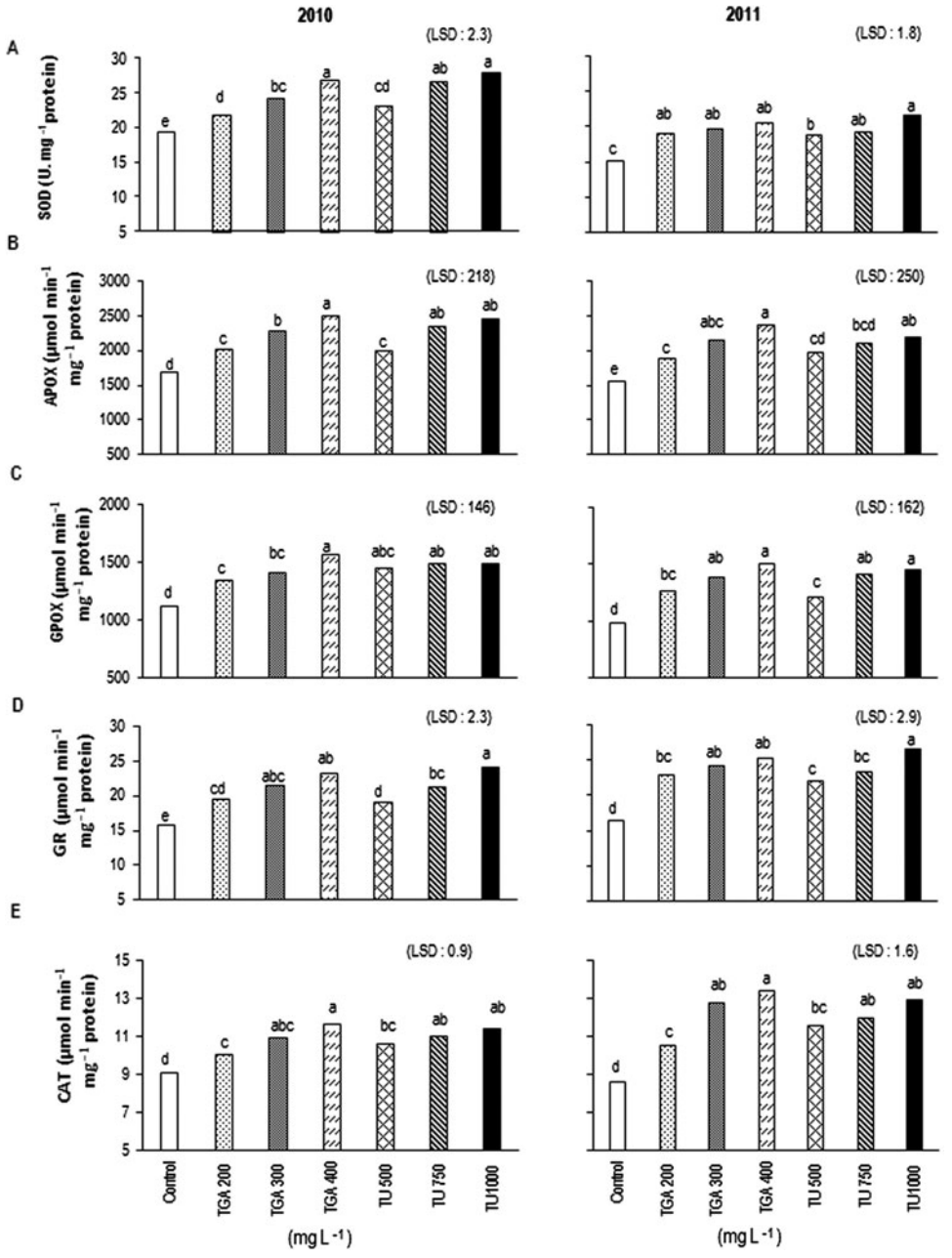

Figure 1. Effects of foliar application of -SH compounds on (a) SOD, (b) APOX, (c) GPOX, (d) GR and (e) CAT antioxidant enzyme activities. Columns with different letters indicate that they differ significantly ($p \leq 0.05$).

Antioxidant enzyme activities

The activities of antioxidant enzymes (CAT, APOX, GPOX, SOD and GR) measured at 50 DAS are presented in Figure 1. Exogenous application of -SH compounds significantly ($p \leq 0.05$) enhanced the activities of antioxidant enzymes.

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Compared with non-treated plants, the -SH-treated plants showed 17–42, 21–51, 23–46, 19–44, 32–57% higher activities for CAT, APOX, GPOX, SOD and GR respectively. The effects of -SH application on antioxidant enzyme activities was chemical- and concentration-specific. For instance, the highest activity of CAT, APOX and GPOX were recorded for TGA 400 mg L^{-1}-treated plants, whereas TU 1000 mg L^{-1}-treated plants showed the highest activity of SOD and GR enzymes.

MDA content and MSI

The MDA content and MSI varied significantly ($p \leq 0.05$) among tested treatments (Figures 2a and b). Application of exogenous -SH compounds significantly ($p \leq 0.05$) decreased MDA content and increased MSI. Averaged across the years, the mean MDA contents for -SH-treated plants were 10 to 19% lower, and MSI was 11 to 18% higher than the untreated plants. The TGA 400 mg L^{-1}-treated plants had the highest MSI and the lowest MDA contents in both the years.

Photosynthetic pigments

The effects of application of -SH compounds on chlorophyll contents and carotenoids are presented in Figures 2c–f. Application of -SH compounds significantly ($p \leq 0.05$) improved the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid during both the years. The -SH-treated plants showed greater increment in chlorophyll content relative to carotenoid content, and for chlorophyll *a* than chlorophyll *b*. Averaged across both the years, -SH application had 23–38, 11–17, 18–30 and 9–16% greater chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents, respectively, compared with untreated plants. The greatest increments in the contents of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) were recorded for 1000 mg L^{-1} TU.

Gas exchange parameters

Effects of application of -SH compounds on gas exchange parameters are presented in Figure 3. The g_s had a range of 394–561 mmol m^{-2} s^{-1}. Averaged across the years, the -SH-treated plants had 17 to 42% greater g_s (Figure 3a) and 11–24% higher E values (Figure 3b) than untreated plants. Averaged across the years, the P_N value varied from 11 to 15 μmol m^{-2} s^{-1}. Exogenous -SH compounds significantly ($p \leq 0.05$) increased P_N, and -SH-treated plants had 1.2 to 1.4 times greater P_N value compared with untreated plants. Among -SH compounds tested, TGA-treated plants showed greater increments in P_N relative to TU-treated plants (Figure 3c). The WUE, measured as the ratio between P_N and E, showed significant ($p \leq 0.05$) response to -SH application, and -SH-treated plants exhibited 10–14% higher values of WUE than that of untreated plants (Figure 3d). The plants treated with 400 mg L^{-1} TGA had the greatest P_N, g_s and E values followed by TU 1000 mg L^{-1}.

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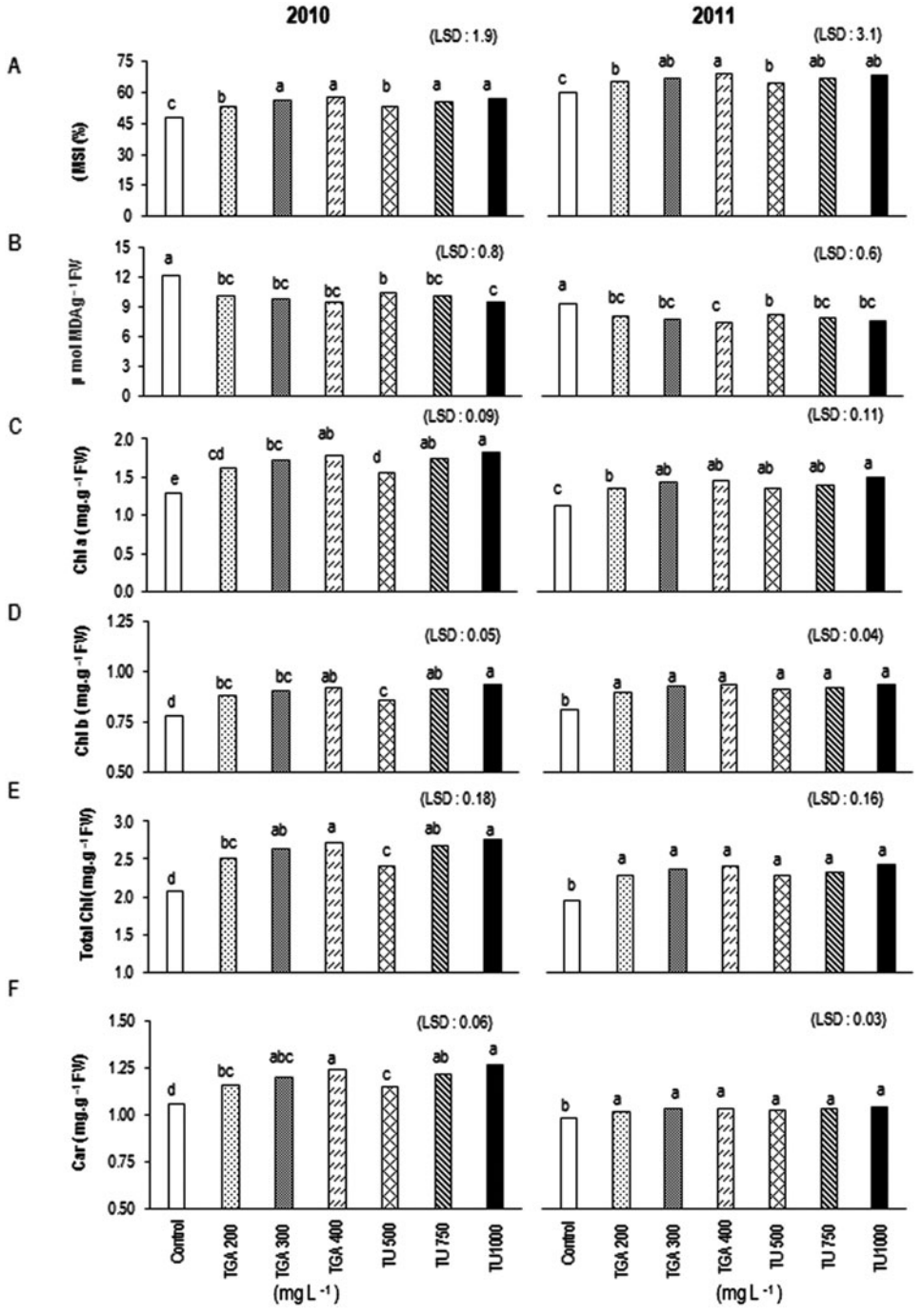

Figure 2. Effects of foliar application of -SH compounds on (a) MSI, (b) MDA content, (c) chlorophyll *a*, (d) chlorophyll *b*, (e) total chlorophyll and (f) carotenoid content. Columns with different letters indicate that they differ significantly ($p \leq 0.05$).

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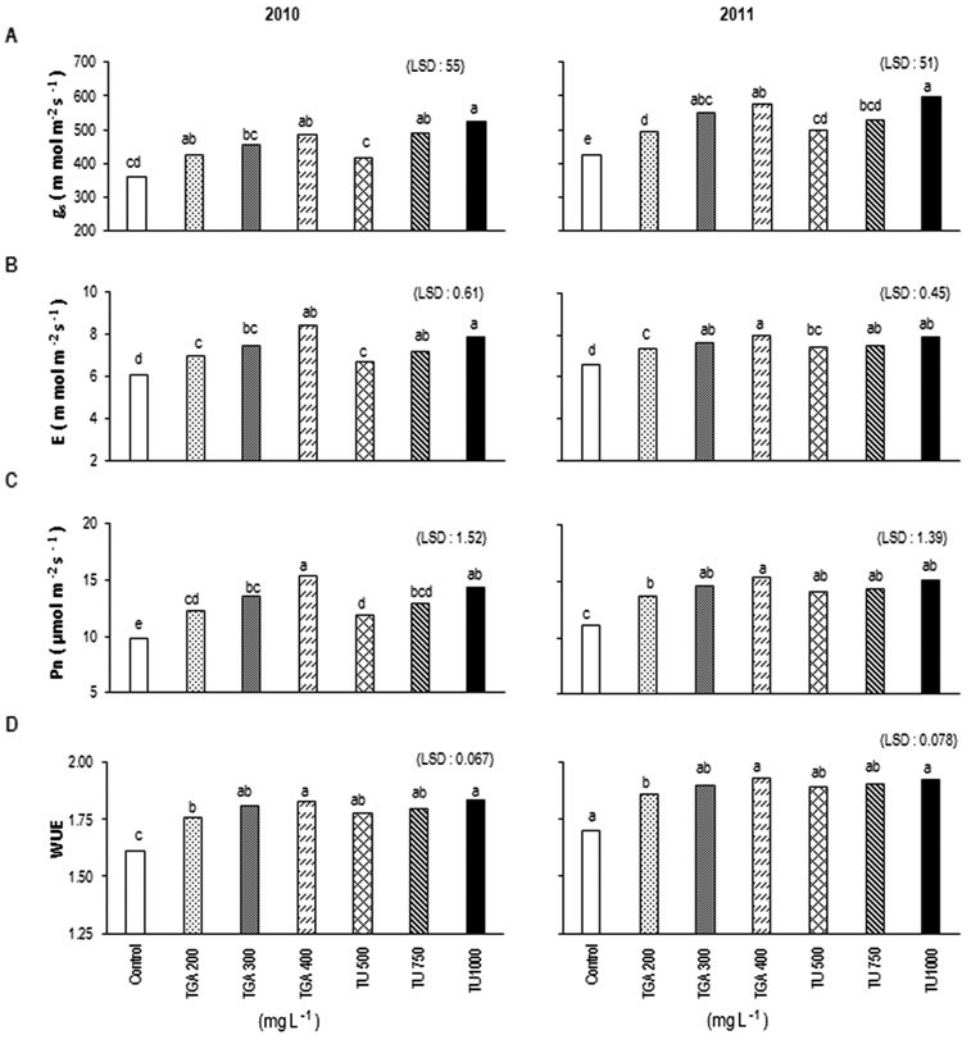

Figure 3. Effects of foliar application of -SH compounds on (a) g_s, (b) E, (c) P_N and (d) WUE. Columns with different letters indicate that they differ significantly ($p \leq 0.05$).

Growth attributes

Foliar application of -SH compounds had significant effects ($p \leq 0.05$) on LAI and DM measured at 50, 60 and 70 DAS during both the years (Table 2). The LAI varied from 1.5 to 2.0, 2.2 to 3.0 and 2.4 to 3.2 at 50, 60 and 70 DAS respectively. Compared with untreated plants, the -SH-treated plants had 16–28, 20–34 and 21–33% higher LAI at 50, 60 and 70 DAS respectively. The -SH application significantly ($p \leq 0.05$) improved DM accumulation. Averaged across both the years, the -SH-treated plants had 12–19, 21–32 and 25–38% higher DM production than untreated plants measured at 50, 60 and 70 DAS respectively.

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Table 2. Effects of foliar application of -SH compounds on leaf area index (LAI) and dry matter (DM) accumulation of clusterbean grown during 2010 and 2011 at Bikaner, Rajasthan, India.

	2010						2011					
	LAI			DM (g plant^{-1})			LAI			DM (g plant^{-1})		
Treatment	50 DAS	60 DAS	70 DAS	50 DAS	60 DAS	70 DAS	50 DAS	60 DAS	70 DAS	50 DAS	60 DAS	70 DAS
Control	1.58^{b}	2.26^{c}	2.37^{c}	8.7^{d}	19.6^{c}	24.2^{c}	1.55^{d}	2.21^{d}	2.33^{c}	8.3^{c}	18.3^{b}	23.9^{c}
$TGA_{200\ ppm}$	1.86^{a}	2.71^{b}	2.88^{b}	9.7^{bc}	24.5^{b}	31.1^{b}	1.78^{c}	2.68^{c}	2.79^{b}	9.4^{b}	21.4^{c}	28.8^{b}
$TGA_{300\ ppm}$	1.92^{a}	2.86^{ab}	3.06^{ab}	10.2^{ab}	26.4^{ab}	34.0^{ab}	1.91^{abc}	2.82^{abc}	2.97^{ab}	9.9^{ab}	22.3^{abc}	30.6^{ab}
$TGA_{400\ ppm}$	1.98^{a}	2.91^{ab}	3.12^{ab}	10.3^{a}	27.2^{a}	35.3^{a}	1.99^{a}	2.95^{ab}	3.09^{a}	9.9^{ab}	23.3^{a}	31.4^{a}
$TU_{500\ ppm}$	1.90^{a}	2.73^{b}	2.87^{b}	9.5^{c}	24.8^{b}	31.4^{b}	1.84^{bc}	2.72^{bc}	2.81^{b}	9.6^{ab}	21.7^{bc}	29.1^{ab}
$TU_{750\ ppm}$	1.96^{a}	2.90^{ab}	3.06^{ab}	10.1^{ab}	26.2^{ab}	33.7^{ab}	1.94^{ab}	2.88^{abc}	3.00^{ab}	9.8^{ab}	22.4^{abc}	30.1^{ab}
$TU_{1000\ ppm}$	2.00^{a}	2.98^{a}	3.17^{a}	10.1^{ab}	27.0^{a}	35.1^{a}	2.01^{a}	3.02^{a}	3.08^{a}	10.0^{a}	23.1^{ab}	31.2^{ab}
$LSD_{(0.05)}$	0.14	0.22	0.26	0.5	2.1	3.1	0.14	0.23	0.25	0.6	1.5	2.4

Values followed by different letters in each column are significantly different at $p \leq 0.05$ according to least significant difference (LSD).
TGA: thioglycolic acid; TU: thiourea.

Table 3. Effects of foliar application of -SH compounds on yield components and clusterbean yield grown during 2010 and 2011 at Bikaner, Rajasthan, India.

	2010					2011				
Treatment	NP (plant^{-1})	NS (pod^{-1})	1000-seed weight (g)	SY (kg ha^{-1})	ABY (kg ha^{-1})	NP (plant^{-1})	NS (pod^{-1})	1000-seed weight (g)	SY (kg ha^{-1})	ABY (kg ha^{-1})
Control	91^{d}	7.1^{e}	29.4^{a}	1006^{c}	3782^{c}	87^{c}	6.9^{c}	29.1^{a}	968^{c}	3652^{c}
$TGA_{200\ ppm}$	107^{c}	7.5^{d}	29.9^{a}	1163^{b}	4318^{b}	100^{b}	7.6^{a}	29.7^{a}	1094^{b}	4107^{b}
$TGA_{300\ ppm}$	111^{abc}	7.9^{bc}	30.4^{a}	1211^{ab}	4631^{ab}	104^{ab}	7.8^{ab}	29.7^{a}	1139^{ab}	4243^{ab}
$TGA_{400\ ppm}$	116^{a}	8.0^{ab}	30.6^{a}	1247^{a}	4781^{a}	106^{a}	7.8^{ab}	30.7^{a}	1151^{a}	4324^{ab}
$TU_{500\ ppm}$	109^{bc}	7.7^{cd}	30.1^{a}	1170^{b}	4536^{ab}	101^{b}	7.6^{b}	29.5^{a}	1113^{b}	4173^{ab}
$TU_{750\ ppm}$	110^{bc}	7.8^{bc}	30.7^{a}	1223^{ab}	4630^{ab}	102^{ab}	7.7^{ab}	29.8^{a}	1129^{ab}	4267^{ab}
$TU_{1000\ ppm}$	114^{a}	8.2^{a}	31.2^{a}	1233^{ab}	4818^{a}	104^{ab}	7.7^{ab}	30.9^{a}	1146^{a}	4358^{a}
$LSD_{(0.05)}$	7	0.2	NS	73	322	5	0.3	NS	63	242

Values followed by different letters in each column are significantly different at $p \leq 0.05$ according to least significant difference (LSD).
TGA: Thioglycolic acid; TU: Thiourea; NP: pod number per plant; NS: seed number per plant; SY: seed yield; ABY: aboveground biomass yield.

Yield components and yields

Effects of application of -SH compounds on yield components (NP, NS and 1000-seed weight) and yields (SY and ABY) are presented in Table 3. Application of -SH compounds had significant effects on NP and NS during both the years. The NP varied from 87 to 114 plant^{-1} and SN ranged from 6.9 to 8.2 per pod. Averaged across both the years, -SH compounds-treated plants had 16–23% higher NP and 8–14% higher NS than untreated plants. The -SH application failed to effect significant difference in 1000-seed weight during either year.

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Table 4. Correlations between dry matter accumulation at 70 DAS ($DM_{70\ DAS}$), malondialdehyde (MDA) content, membrane stability index (MSI), total chlorophyll content (TCHL), activity of antioxidant enzymes (CAT, SOD, APOX, GPOX and GR) and gas exchange parameters.

	$DM_{(70\ DAS)}$	MDA	MSI	TCHL	CAT	SOD	APOX	GPOX	GR	P_N	g_s	E
$DM_{(70\ DAS)}$	1.00†											
MDA	−0.29^{NS}	1.00										
MSI	0.35^{NS}	−0.97**	1.00									
TCHL	0.98**	−0.32^{NS}	0.48*	1.00								
CAT	0.43^{NS}	−0.72*	0.64*	0.29^{NS}	1.00							
SOD	0.94**	−0.42^{NS}	0.57*	0.97**	0.17^{NS}	1.00						
APOX	0.94**	−0.28^{NS}	0.08^{NS}	0.88**	0.64*	0.83**	1.00					
GPOX	0.91**	−0.34^{NS}	0.11^{NS}	0.84**	0.64*	0.80**	0.92**	1.00				
GR	0.47^{NS}	−0.75*	0.63*	0.36^{NS}	0.90**	0.25^{NS}	0.66*	0.62*	1.00			
P_N	0.51*	−0.72*	0.59*	0.48^{NS}	0.88**	0.27^{NS}	0.71*	0.61*	0.93**	1.00		
g_s	0.19^{NS}	−0.86**	0.80**	0.07^{NS}	0.88**	−0.01^{NS}	0.45^{NS}	0.39^{NS}	0.92**	0.83**	1.00	
E	0.63*	−0.55*	0.38^{NS}	0.53*	0.75*	0.43^{NS}	0.80**	0.66*	0.84**	0.96**	0.70*	1.00

†Pearson correlation coefficient.

NS: non-significant; *significant at $p \leq 0.05$; **significant at $p \leq 0.01$.

DM $_{(70\ DAS)}$: dry matter at 70 DAS; CAT: catalase; SOD: superoxide dismutase; APOX: ascorbate peroxidase; GPOX: guaiacol peroxidase; GR: glutathione reductase; P_N: net photosynthetic rate; g_s: stomatal conductance; E: transpiration rate.

Seed yield varied from 1006–1247 kg ha^{-1} and 968–1151 kg ha^{-1} during 2010 and 2011 respectively. Application of -SH compounds significantly ($p \leq 0.05$) increased SY in both the years (Table 3). Averaged across the years, application of -SH compounds gave 14 to 22% higher SY than control (untreated) plants. Compared with untreated plants, TGA 400 mg L^{-1}-, TU 1000 mg L^{-1}-, TGA 300 mg L^{-1}-, TU 750 mg L^{-1}-, TU 500 mg L^{-1}- and TGA 200 mg L^{-1}-treated plants gave 212, 202, 190, 188, 154 and 142 kg ha^{-1} higher SY respectively. ABY varied from 3652 to 4818 kg ha^{-1}. Averaged across both the years, the -SH-treated plants had 495 to 871 kg ha^{-1} greater ABY than control.

Correlation among measured parameters

Correlation coefficients among DM (at 70 DAS), MDA content, MSI, total chlorophyll content, antioxidant enzyme activities and gas exchange parameters are shown in Table 4. DM was positively correlated with the activities of antioxidant enzymes, TCHL content, gas exchange parameters, and negatively correlated with MDA content. MDA content was negatively correlated with most of the measured parameters. P_N had significant positive correlation with the activities of CAT, APOX, GPOX and GR. Overall, the results indicate that activities of antioxidant enzymes are positively correlated with MSI, total chlorophyll content and gas exchange parameters, and negatively correlated with MDA content.

DISCUSSION

Drought is one of the most serious production constraints for world agriculture (Mir *et al.*, 2012). The exogenous application of stress alleviating substances has been

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advocated as an effective option to achieve sustainable yields under water deficit conditions. An important requirement for determining efficacy of stress alleviating substances in plants is a proper comparison of biochemical parameters among the plants treated with these chemicals.

Exposure of plants to environmental stress conditions leads to the generation of ROS, which causes oxidative damage. The equilibrium between production and scavenging of ROS may be perturbed by a number of adverse abiotic stress factors (Apel and Hirt, 2004). Our results showed significant enhancement in the activity of antioxidant enzymes with application of exogenous -SH compounds (Figure 1).

Enhancement of antioxidant enzymes with the application of exogenous -SH compounds might be attributed to increased synthesis of enzymes (i.e., glutathione is a thiol containing compound, which is an essential cofactor for GPOX) due to supply of thiol (-SH) containing compounds (Loggini *et al.*, 1999). Furthermore, higher photosynthetic activity (Figure 3) for -SH-treated plants increases soluble sugar content, which could stabilize the structure and hence result in higher activity of antioxidant enzymes (Chaves *et al.*, 2003). The observed positive correlations among the activities of SOD, CAT, APOX, GPOX and GR in our study (Table 4) suggested that increase in SOD activity was accompanied by increase in the activities of other enzymes as a result of high demand of quenching H_2O_2. This increased antioxidant production, lowered the ROS-based damages in plant system, as is evident from the negative correlation between antioxidant enzymes activities and MDA content (Table 4).

Decrease in MDA content (Figure 2b) and increase in MSI (Figure 2a) observed under -SH treatments in the present study could be attributed to decreased lipid peroxidation due to enhanced activities of antioxidant enzymes (Figure 1). An increased activity of antioxidant enzymes has been correlated with decreased lipid peroxidation under stress conditions (Anjum *et al.*, 2012), and this relationship was confirmed by our correlation analysis (Table 4). Besides antioxidant protection, the intracellular and extracellular redox states of thiols play a critical role in the stabilization of protein structure and function, regulation of enzyme activity and control of transcription factor activity (Ramaswamy *et al.*, 2007).

Decrease in chlorophyll content under water deficit stress is attributed to the oxidation of chlorophyll and damaged ultra-structure of chloroplasts (Anjum *et al.*, 2011). Our results indicated that application of -SH compounds significantly enhanced chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content in leaves of clusterbean under water deficit condition (Figures 2c–f). These results are in agreement with the findings of Liu *et al.* (2002), who observed that application of TU reduced the rate of chlorophyll degradation during senescence; this may be responsible for the maintenance of higher chlorophyll concentration as reported in wheat (Sahu and Singh, 1995), and in clusterbean (Burman *et al.*, 2004). This appears logical because -SH compounds (i.e., thiourea) exhibit cytokinin-like activity, and are known to delay leaf senescence (Vassilev and Mashev, 1974).

Recent studies have suggested that both diffusive limitation through stomatal closure and non-stomatal limitation (such as oxidative damage to chloroplast) are responsible for decline in photosynthesis under drought stress (Prasad, 2008). Our results indicated that -SH application improved photosynthetic parameters (P_N, g_s and E) compared with non-treated plants. Among the studied parameters, the -SH application had the greatest improvements in P_N followed by g_s and E (Figure 3). Higher P_N for -SH-treated plants might be explained by the higher content of photosynthetic pigments (Figure 2) and less water deficit-induced oxidative damage to metabolic activity reflected by higher activity of antioxidant enzymes (Figure 1) along with increasing flow of CO_2 via higher g_s (Figure 3a). This is confirmed by a correlation study, which indicated that P_N was positively correlated with photosynthetic pigment contents, antioxidant enzymes activity, chlorophyll content and g_s (Table 4). D'Souza *et al.* (2009) found that -SH compounds help plants to maintain photosynthetic efficiency and delay chlorophyll degradation, besides providing antioxidant protection and stability in protein structure.

Plants treated with foliar application of -SH compounds had significantly greater growth measured in terms of LAI and DMA (Table 2). LAI, which denotes extent of radiation capture by crops under field conditions, decreases under drought. Broadly, Drought-induced reduction in LAI is ascribed to reduction in cell wall turgor and photosynthesis. Reduction in DM under drought is mainly ascribed to reduce LAI, which results in reduced light interception (Nam *et al.*, 1998). The better LAI of clusterbean treated with -SH compounds may be explained by their ability to maintain higher cell turgor and photosynthesis under moisture deficit conditions. Burman *et al.* (2004) and Garg *et al.* (2006) postulated that -SH compounds enhanced photosynthesis, which increased sap production in the leaf lamella, and resulted in the maintenance of relative water content (RWC) and water potential (WP) in the leaf, and hence higher cell turgor. The higher LAI coupled with higher P_N might be responsible for enhanced DM accumulation (DMA) of -SH compound-treated plants.

In the present study, exogenous application of -SH compounds led to significantly higher numbers of pods and seeds per pod compared with untreated plants (Table 3). The -SH compounds can protect cell membrane and negative effects of ROS during stress, which can lead to greater production of assimilates (indicated by greater DM) and improved translocation of assimilates and apportioning to sinks (Srivastava *et al.*, 2008). Thus, improvement in yield components with -SH compounds may be explained by the ability of -SH compounds to increase DM production, better translocation of assimilates and apportioning of DM to sinks.

Drought mainly influences yield by limiting seed numbers by either influencing the amount of DM produced by the time of flowering or by directly influencing pollen or ovule function, which leads to decreased seed-set. Secondarily, drought influences seed filling mainly by limiting the assimilate supply, leading to smaller seed size and lower yields (Prasad, 2008). Significant improvement in seed yields with -SH application in the present study could be attributed to significant improvement in yield attributes (pod number and seed per pod) due to greater photosynthesis and better

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partitioning of photosynthates. The enhancement of photosynthesis (Nathawat *et al.*, 2007; Ramaswamy *et al.*, 2007) and better partitioning of photosynthates to sink for various crop species treated with -SH compounds have been reported earlier under abiotic stress conditions (Sahu and Singh, 1995).

CONCLUSIONS

In conclusion, our results indicate that exogenous application of -SH compounds ameliorate clusterbean crop performance in a water deficit environment. This ameliorative effect was associated with the alteration of physio-biochemical processes such as increase in antioxidant enzyme activities, photosynthetic pigment content, gas exchange parameters and membrane stability, and decrease in lipid peroxidation. The study shows that foliar spray of -SH compounds (TU and TGA) can help to increase clusterbean yield under a water deficit environment. The -SH compounds can be considered as a potential growth regulator for improving crop growth and yield under limited soil moisture conditions.

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REFERENCES

Anjum, S. A., Saleem, M. F., Wang, L. C., Bilal, M. F. and Saeed, A. (2012). Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. *Australian Journal of Crop Science* 6:576–583.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C. and Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research* 6:2026–2032.

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* 55:373–399.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24:1–15.

Ashraf, M. (2010). Inducing drought tolerance in plants: recent advances. *Biotechnology Advances* 28:169–183.

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

Blum, A. (2005). Drought resistance, water-use efficiency and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* 56:1159–1168.

Burman, U., Garg, B. K. and Kathju, S. (2004). Interactive effects of thiourea and phosphorus on cluster bean under water stress. *Biologia Plantarum* 48:61– 65.

Cakmak, I. and Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* 83:463–468.

Chance, B. and Maehly, A. C. (1955). Assay of catalase and peroxidases. *Methods Enzymology* 2:764–755.

Chaves, M. M., Maroco, J. P. and Pereira, J. S. (2003). Understanding plant responses to drought from genes to the whole plant. *Functional Plant Biology* 30:239–264.

D'Souza, S. F., Nathawat, N. S., Nair, J. S., Radhakrishna, P., Ramaswamy, N. K., Singh, G. and Sahu, M. P. (2009). Enhancement of antioxidant enzyme activity and primary photochemical reactions in response to foliar applications of thiols in water stress in pearl millet. *Acta Agronomica Hungarica* 57:21–31.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* 29:185–212.

Garg, B. K., Burman, U. and Kathju, S. (2006). Influence of thiourea on photosynthesis, nitrogen metabolism and yield of cluster bean (*Cyamopsis tetragonoloba* L.) under rain-fed conditions of Indian arid zone. *Plant Growth Regulation* 48:237–245.

Gomez, K. A. and Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research*. New York, NY: Willy.

Gresta, F., Sortino, O., Santonoceto, C., Issi, L., Formantici, C. and Galante, Y. M. (2013). Effects of sowing times on seed yield, protein and galactomannans content of four varieties of guar (*Cyamopsis tetragonoloba* L.) in a Mediterranean environment. *Industrial Crops and Products* 41:46–52.

Hasanuzzaman, M., Hossain, M. A., da Silva, J. A. T. and Fujita, M. (2012). Plant responses and tolerance to abiotic oxidative stress: antioxidant defences is a key factors. In *Crop Stress and its Management: Perspectives and Strategies*, 261–316 (Eds V. Bandi, A. K. Shanker, C. Shanker and M. Mandapaka). Berlin, Germany: Springer.

Hiscox, J. D. and Israelstam, G. F. (1979). A method for extraction of chloroplast from leaf tissue without maceration. *Canadian Journal of Botany* 57:1332–1334.

Liu, P., Li, H. Y., Shang, Y. L. and Qi, F. G. (2002). Effects of N-(1-naphtalene acetyl)-N'-(2-carboxy phenyl) thiourea (NCT) on senescent physiological activities of wheat. *Acta Agriculturae Boreali Sinica* 17:33–36.

Loggini, B., Scartazza, A., Brugnoli, E. and Navari-Izzo, F. (1999). Antioxidative defence system, pigments composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119:1091–1099.

Mir, R. R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R. K. (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theoretical and Applied Genetics* 125:625–645.

Moller, I. M., Jensen, P. E. and Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology* 58:459–481.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiology* 22:867–880.

Nam, N. H., Subbaroa, G. V., Chauhan, Y. S. and Johansen, C. (1998). Importance of canopy attributes in determining dry matter accumulation of pigeonpea under contrasting moisture regimes. *Crop Science* 38:955–961.

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

Nogueira, A., Martinez, C. A., Ferreira, L. L. and Prado, C. H. B. A. (2004). Photosynthesis and water use efficiency in twenty tropical tree species of differing succession status in a Brazilian reforestation. *Photosynthetica* 42:351–356.

Pathak, R., Singh, M. and Henry, A. (2011). Genetic diversity and interrelationship among cluster bean (*Cyamopsis tetragonoloba*) genotypes for qualitative traits. *Indian Journal of Agricultural Science* 81:402–406.

Prasad, P. V. V., Staggenborg, S. A. and Ristic, Z. (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In *Response of Crops to Limited Water: Understanding and Modelling Water Stress Effects on Plant Growth Processes*. Advances in Agricultural Systems Modeling Series 1. 301–355 (Eds L. R. Ahuja, V. R. Reddy, S. A. Saseendran and Q. Yu) Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Ramaswamy, N. K., Nathawat, N. S., Nair, J. S., Sharma, H. R., Kumawat, S. M., Singh, G., Sahu, M. P. and 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.

Rikin, A., Atsmon, D. and Gitler, C. (1979). Chilling injury in cotton (*Gossypium hirsutum* L.) prevention by abscisic acid. *Plant Cell Physiology* 20:1537–1546.

Sahu, M. P. and Singh, D. (1995). Role of thiourea in improving productivity of wheat (*Triticum aestivum* L.). *Plant Growth Regulation* 14:169–173.

Sairam, R. K., Rao, K. V. and Srivastava, G. C. (2002). Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* 163:1037–1046.

Shaedle, M. and Bassham, J. A. (1977). Chloroplast glutathione reductase. *Plant Physiology* 59:1011–1012.

Srivastava, A. K., Nathawat, N. S., Ramaswamy, N. K., Sahu, M. P., Singh, G., Nair, J. S., Paladi, R. K. and D'Souza, S. F. (2008). Evidence for thiol-induced enhanced *in situ* translocation of ^{14}C – sucrose from source to sink in *Brassica juncea*. *Environmental and Experimental Botany* 64:250–255.

Vassilev, G. N. and Mashev, N. P. (1974). Synthesis, chemical structure and cytokinin-like activity of some derivatives of P-phenyl-N' like or aryl thiourea and their influence on nitrogen metabolism in barley seedlings. *Biochemie und Physiologie der Pflanzen* 165:467–458.