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Influence of Plant Bio-regulators on Physiological Traits, Gas Exchange and Yield of Clusterbean (*Cyamopsis tetragonoloba* L. Taub) under Water Stress

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Abstract: Clusterbean (*Cyamopsis tetragonoloba* L.Taub) is the most important summer legume crop grown in hot arid regions. However, little is known of its morphological, physio-biochemical responses, and yield traits to drought and sulphydryl compounds (-SH compounds) application. Drought had significant negative effects on growth and metabolism, as revealed by reduced leaf area, net assimilation rate, leaf relative water content photosynthetic rate, photosynthetic pigments and weakened yield traits. A field experiment was conducted for two consecutive years to investigate the effect of foliar application of two plant bio-regulators (PBR's) applied at different concentrations namely, thioglycollic (TGA) acid at 200 mg L^{-1}, 300 mg L^{-1} and 400 mg L^{-1}; thiourea at 500 mg L^{-1}, 750 mg L^{-1} and 1000 mg L^{-1} on morpho-physiological traits and yield parameters. Foliar application of PBRs at different concentrations increased 16-35%, 11-20%, 9-30%, 8-39% and 14-21% higher growth attributes, water relations, photosynthetic pigments, gas exchange parameters, and seed yield, respectively. Although eliciting behavior of bio-regulators improved morpho-physiological attributes against drought stress condition, the application of TGA 400 mg L^{-1} and TU 1000 mg L^{-1} recorded maximum net assimilation rate, leaf area, RWC, total chlorophyll, carotenoid content, pods, 100 seed weight and seed yield over control.

Key words: Net assimilation rate, photosynthetic rate, stomatal conductance, relative water content, photosynthetic pigment, water potential, water use efficacy, yield.

Drought stress is one of the main environmental factors limiting plant growth and yield. It is the most prevalent cause of crop yield loss due to an increase in temperature and a decrease in water availability that deviates from the optimal condition for plant life. The responses of plants to drought stress depend on species, genotype, the length and severity of water deficit, the age and stage of development (Bray, 1997). Water deficit leads to the perturbation of most of the physiological and biochemical processes and consequently reduces plant growth and yield (Boutraa, 2010). The productivity of the crop may be related to physiological attributes like photosynthetic rate, photosynthetic pigments, leaf relative water content and leaf water potential. Higher photosynthetic efficiency and RWC indicates better growth and development, which is turn depend on leaf area. Rapid early growth and maintenance of RWC at reasonably higher level with higher photosynthetic efficiency during reproductive phase greatly influences the yield (Haloi and Baldev, 1986). Growth analysis is a useful tool in studying the complex interactions between plant growth and the environment, describing and interpreting physiological response (Nathawat *et al.*, 2007a). Food productivity is decreasing due to detrimental effects of various biotic and abiotic stresses; therefore minimizing these losses is a major area of concern to ensure food security under changing climate. Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals or high salinity, severely impair plant growth and productivity worldwide (Anjum *et al.*, 2011). Drought being the most important environmental stress severely impairs plant growth and development, limits plant production and the performance of crop plants more than any other environmental factor (Shao *et al.*, 2009). Available water resources for successful crop productions have been decreasing in recent years. The susceptibility

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of plants to drought stress varies in dependence of stress degree, different accompanying stress factors, plant species and their developmental stages (Demirevska *et al*., 2009). Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses.

Clusterbean (*Cyamopsis tetragonoloba* L.) is a legume species cultivated mainly in India and, to a lesser extent in Pakistan and the USA. Guar gum and its derivatives are extensively used as an emulsifier, thicker, strengthener and stabilizer in a wide range of industrial activities such as food, paper printing, textiles, cosmetics and oil industries (Mudgil *et al*., 2014). India is a major producer of cluster bean and contributes to 80% of the world's total production of cluster bean (Pathak *et al*., 2011). 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.

During drought stress, plant tendency is to reduce its yield and goes into survival mode which ultimately reduce plant yield. Many methods have been used to mitigate drought stress such as transformation of drought resistant genes to a host plant and cultivation of drought tolerant crop. However, methods need high investment technology and long time or just certain plant must be cultivated accordingly to edaphic and environmental condition. Plant bio-regulators are biochemical compounds stimulates plant growth and productivity when applied, even in small quantities at appropriate plant growth stages. These are being extensively used in agriculture to enhance the productivity in crops (Sahu and Solanki, 1991). Their central role in plant growth and development is through nutrient allocation and source-sink transitions while most of the PBRs stimulate redox signaling under abiotic stress conditions (Nathawat *et al*., 2018; Srivastava *et al*., 2008). Since climate change and degrading natural resources are projected to amplify the stresses, particularly soil moisture deficit, high temperature and soil salinity, PBRs are likely to play a crucial role in plant growth regulation. Research efforts so far have centered on the crop and agro-ecosystem specificity, optimal doses and schedule of their application for optimizing crop yields under stress conditions. There is a lack of comprehensive information available in the literature on the effect of bio-regulators (TGA and TU) on growth attributes, water status, photosynthetic efficiency and yield traits of clusterbean grown under climatic condition of hot arid region of India. The present study was carried out, therefore to investigate the response of bio-regulators on physio-biochemical changes, yield attributes and yield of clusterbean under drought stress. However, to the best of our knowledge, information regarding the effect of foliar application of bioregulators on the growth and development of clusterbean under water stress is less available. Therefore, the purpose of this study is to understand whether application of bioregulators raises photosynthetic efficiency and productivity of clusterbean under water stress.

Materials and Methods

An experiment was conducted during two kharif seasons (July-October) in the two consecutive years at the ICAR-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 area is characterized as arid with hot and dry spring-summer from April to June, hot and humid summer from July to September. The average annual rainfall is about 287 mm, most of which is received during the monsoon period (July to September). The total amount of rainfall received during the crop seasons (from July to October) was 281 mm and 368 mm in 1st year and 2nd year, respectively. The soil of the experimental field was sandy in texture having pH 8.4, with 0.16% organic carbon, 9.8 kg P ha^{-1} and 236.0 kg K ha^{-1}. The experiment comprised seven treatments *e.i.* water spray, TGA 200 mg L^{-1}, TGA 300 mg L^{-1}, 400 TGA mg L^{-1}, TU 500 mg L^{-1}, TU 750 mg L^{-1} and TU 1000 mg L^{-1} and laid out in a randomized complete block design with three replications. Two foliar sprays of the sulphydryl compounds were applied at vegetative and pre-flowering stages of the crop. Size of each plot was 5.0 m × 3.5 m with 2 m gap in between. Each plot was bordered with an earth dike 30 cm in height. Overall, there were 21 plots for the whole experiment. Treatments were randomly assigned in each replication during first year of the study. Thereafter, a particular treatment was assigned with the same plot unit during the following year. Cluster bean (cultivar RGC-1003) was sown on last week of July during both the years.

Growth indices

Plant growth characteristics i.e. leaf area (LA), leaf area duration (LAD) and net assimilation rate (NAR) were measured by harvesting three plants randomly from each plot at a 10-days interval starting from 50-70 days after sowing (DAS).

The total leaf area per plant was measured by portable leaf area meter model (LI-3000 LI-COR, USA) and expressed as cm^2 $plant^{-1}$.

Leaf area duration (LAD) was calculated following the protocol devised by Hunt (1978), using the following equation and expressed as days

$$LAD = (LA\ I_1 + LA\ I_2)\ (t_2 - t_1) / 2$$

NAR was calculated by Williams (1948) and expressed as mg cm^{-2} day^{-1}.

$$NAR = W_2 - W_1 / t_2 - t_1 \times Log_e\ LA_2 - Log_e\ LA_1 / LA_2 - LA_1$$

LA_1 and LA_2 are the leaf area (cm^2) and $W_1$and W_2 are total dry weight of a plant (g) at time interval t_1 and t_2 (days), respectively.

Water relation

Leaf samples were taken at flowering stage (50 DAS) from two plants per plot. Completely developed third and fourth leaves from the top of the plant were used for all measurement. Leaves were collected on ice between 9.30-10.30 h and taken to the laboratory, washed with distilled water and excess water removed.

Relative water content (RWC) of leaf samples was determined as described by Barrs and Weatherley (1962). To determine the RWC, leaves were collected, immediately weighed (fresh wt.), rehydrated for 4 h (turgid wt.) and subsequently oven-dried at 85°C to constant mass (dry wt.). The RWC was determined by the following formula and expressed as in percent:

$$RWC = [(Fresh\ wt. - Dry\ wt.) / (Turgid\ wt. - Dry\ wt.)] \times 100$$

Leaf water potential (ψ_w) of fully expanded youngest leaves was measured during 9.30-11.30 h by WP 4 Dew-Point Potential Meter.

Photosynthetic pigment contents

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 amounts of chlorophyll *a*, *b* and carotenoids were determined spectrophotometrically {Double Beam UV Visible Spectrophotometer (Model: 2203) Systronics}, by reading the absorbance at 645, 663 and 470 nm, respectively (Arnon, 1949).

Antioxidant enzyme

Leaf samples (0.5 g fresh weight) were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetraaceatic acid (EDTA) and 1% (w/v) polyvinyl polypyrolidone (PVP). The homogenate was filtered through four layers of cheese cloth 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 the GST assay. GST activity was measured as per Mannervik and Guthenberg (1981) by following the 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 m M^{-1} cm^{-1}).

Gas-exchange parameters

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

Yield attributes and yield

Yield components, *i.e.* pod m^{-2}, seed per m^{-2} and 100-seed weight (SW) were recorded for all the plants from the central 2 × 2 -m area of each plot at harvest. At the maturity stage of the crop 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. Yields of seed and straw were 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) at ά = 0.05.

Results and Discussion

Growth indices

Drought stress is the primary abiotic stress to plant growth, especially under arid and semi-arid conditions. Exogenous application of PBR's was significantly (P≥0.05) improved the growth in terms of LA, LAD and NAR measured at 50, 60 and 70 DAS during both the years (Table 1). During the different growth stages, growth parameters mean values variations were recorded as: LA between 542-1111 cm^{-2}; LAD between 15.5-30.5 days and NAR between 1.65-8.16 mg cm^{-2} d^{-1}. Compared with untreated plants had 16-28%, 20-34% and 21-33% higher leaf area at 50, 60 and 70 DAS respectively. A reduction in leaf area is generally ascribed to the suppression of leaf expansion, as cells lose the turgidity necessary for this process and the declining photosynthetic rate diminishes the availability of photosynthates to build new cells (Rocker *et al*., 1995). Other possible reason for reduced growth in drought stressed plant

Table 1. Effects of foliar application of sulphydryl compounds on leaf area (LA), leaf area duration (LAD) and net assimilation rate (NAR) of cluster bean

Treatments	1st Year						
	Leaf area (cm^{-2})			LAD (Days)		NAR ($mg. cm^{-2}.d^{-1}$)	
	50 DAS	60 DAS	70 DAS	50-60DAS	60-70DAS	50-60 DAS	60-70 DAS
Control	555±35^{b}	792±34^{c}	829±36^{c}	15.8±1.4^{b}	22.6±1.2^{c}	1.65±0.09^{c}	5.67 ± 0.55
TGA 200 mg L^{-1}	653±30^{a}	949±27^{b}	1008±42^{b}	18.6±1.1^{a}	27.1±0.9^{b}	1.88±0.11^{b}	6.80 ± 0.71
TGA 300 mg L^{-1}	674±36^{a}	1000±37^{ab}	1073±50^{ab}	19.2±1.2^{a}	28.6±1.4^{ab}	1.97±0.08^{ab}	7.36 ± 0.65
TGA 400 mg L^{-1}	692±30^{a}	1019±35^{a}	1092±55^{ab}	19.8±1.1^{a}	29.1±1.1^{ab}	2.01±0.08^{a}	7.77 ± 0.55
TU 500 mg L^{-1}	665±32^{a}	956±44^{b}	1006±45^{b}	19.0±1.0^{a}	27.3±1.4^{b}	1.92±0.12^{ab}	6.73 ± 0.71
TU 750 mg L^{-1}	685±36^{a}	1014±41^{ab}	1070±62^{ab}	19.6±1.2^{a}	29.0±1.1^{ab}	1.95±0.12^{ab}	7.21 ± 0.50
TU 1000 mg L^{-1}	701±27^{a}	1043±31^{a}	1111±43^{a}	20.0±0.6^{a}	29.8±0.9^{a}	1.98±0.13^{ab}	7.55 ± 0.61
LSD (0.05)	49	75.6	91.4	1.4	2.2	0.12	0.64

Treatments	2nd Year						
	Leaf area (cm^{-2})			LAD (Days)		NAR ($mg. cm^{-2}.d^{-1}$)	
	50 DAS	60 DAS	70 DAS	50-60 DAS	60-70 DAS	50-60 DAS	60-70 DAS
Control	542±32^{d}	774 ±45^{c}	816±39^{c}	15.5±0.91^{d}	22.1±1.3^{d}	1.98± 0.08^{c}	6.18±0.25^{c}
TGA 200 mg L^{-1}	622±22^{b}	937 ±49^{b}	977±32^{b}	17.8±0.63^{c}	26.8±1.4^{c}	2.29± 0.10^{b}	7.27±0.22^{b}
TGA 300 mg L^{-1}	669±37^{bc}	988±31^{ab}	1040±56^{ab}	19.1±1.06^{abc}	28.2±0.9^{abc}	2.40±0.09^{ab}	7.90±0.47^{a}
TGA 400 mg L^{-1}	696±21^{a}	1032±46^{a}	1082±74^{a}	19.9±0.60^{a}	29.5±1.3^{ab}	2.50±0.07^{a}	8.16±0.45^{a}
TU 500 mg L^{-1}	645±30^{bc}	951 ±26^{b}	984±53^{b}	18.4±0.86^{bc}	27.2±0.7^{bc}	2.29± 0.05^{b}	7.21±0.30^{b}
TU 750 mg L^{-1}	677±19^{ab}	1008±52^{ab}	1050±63^{ab}	19.3±0.54^{ab}	28.8±1.5^{abc}	2.37±0.06^{ab}	7.63±0.24^{ab}
TU 1000 mg L^{-1}	705±25^{a}	1058±37^{a}	1078±35^{a}	20.1±0.71^{a}	30.5±1.1^{a}	2.47±0.11^{a}	7.96±0.34^{a}
LSD (0.05)	48.7	79.4	87.5	1.39	2.27	0.15	0.58

†Values followed by the different letters in each column are significantly different at P ≤ 0.05 according to LSD.
TGA : Thioglycollic acid; TU: Thiourea; Leaf area: LA; LAD: Leaf are duration; NAR: Net assimilation rate.

is a water deficiency that interrupts water flow from soil to the xylem and surrounding cells, which decreases the mobility of available ions, nutrient availabilities and soil microbial activities (Nonami, 1998). Averaged across both the years, the bio-regulators treated plants had 15-24% and 18-35% higher NAR than untreated plants measured at 50-60 and 60-70 DAS growth intervals respectively. The application of TGA and TU also recorded significantly higher NAR than control and increase was found to be 35% and 31% with TGA 400 mg L^{-1} and TU 1000 mg L^{-1} foliar application respectively. The improvement in physiological indices with PBRs might be associated with increased cell division and elongation by virtue of increased photosynthetic efficiency due to improved chlorophyll content and better developed assimilating apparatus and increased dry matter accumulation at growth stages (Sharma *et al.*, 2008).

Water relation

Water relations in plants are important for maintaining their normal growth and metabolism when challenged by drought. The ability to maintain higher water potential is one of the most imperative challenges faced by plant species. Leaf relative water content (LRWC) is therefore considered an effective and reliable indicator for measuring the drought tolerance of plants. The LRWC and water potential (ψ_w) varied significantly (P≥0.05) among tested treatments (Fig. 1). Application of exogenous sulphydryl compounds significantly improved water relation parameters as compared to untreated control. Application of PBR's treated plants had 14-18% higher LWRC than control. The application of TU 1000 mg L^{-1} treated plant had the highest LRWC and ψ_w in both the years. The exogenous applied sulphydryl compounds increased the LRWC and ψ_w under drought stress which may be associated with the higher accumulation of osmolytes though improving its osmotic adjustment. The higher production of photosynthates due to higher contents of photosynthetic pigments and photosynthetic rate (Table 2) under exogenous application of sulphydryl compounds might be an explanation for higher LRWC and ψ_w with these treatments over control.

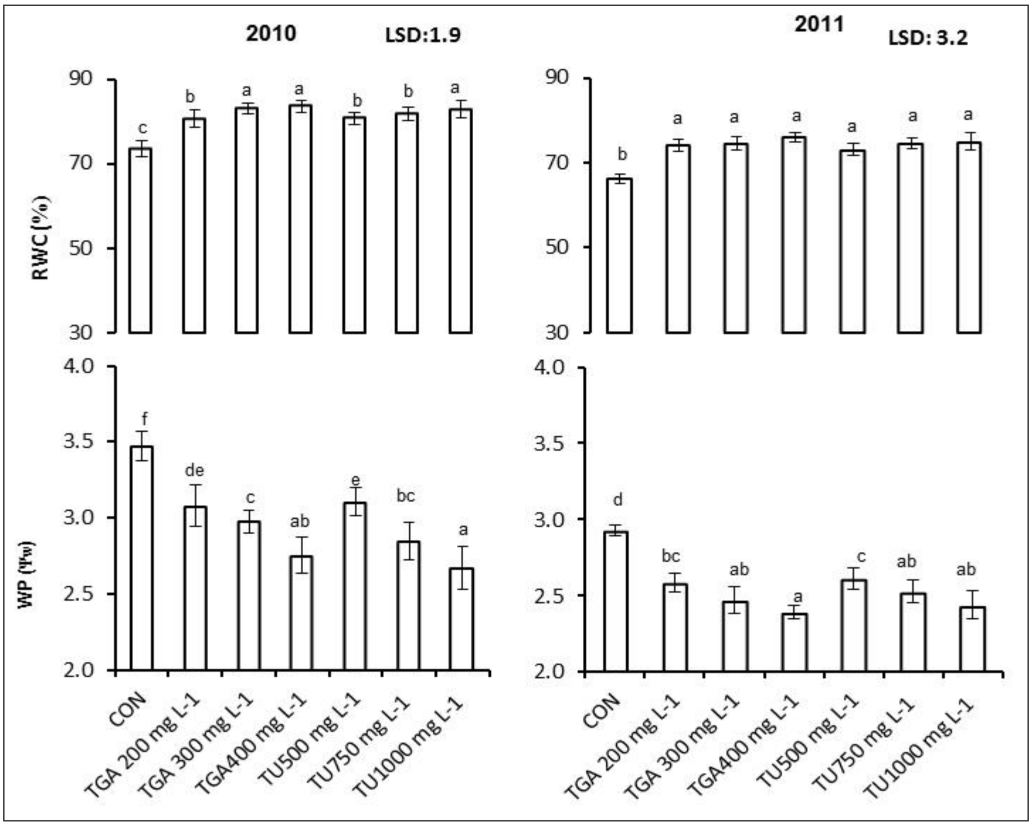

Fig. 1 Effect of -SH compounds on A relative water content (RWC), and B water potential (WP) on clusterbean. Vertical bars represent standard errors. Different small letters indicated that the means are significantly different (p ≤ 0.05).

Table 2. Effects of foliar application of sulphydryl compounds on net photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency of cluster bean at post flowering stage

Treatment	1st Year			
	P_N (μ mol m^{-2} s^{-1})	g_s (m mol m^{-2} s^{-1})	E (m mol m^{-2} s^{-1})	WUE
Control	6.53± 0.38^{d}	320.7± 25.4^{d}	5.90 ±0.15^{d}	1.11±0.015^{b}
TGA 200 mg L^{-1}	7.80 ± 0.36^{c}	403.1 ±26.1^{c}	6.44 ±0.13^{c}	1.21 ±0.023^{a}
TGA 300 mg L^{-1}	8.16 ± 0.30^{bc}	424.8 ±29.6^{bc}	6.64 ±0.23^{bc}	1.23 ±0.012^{a}
TGA 400 mg L^{-1}	8.83 ± 0.29^{a}	481.5 ±24.4^{a}	7.06 ±0.10^{a}	1.25 ±0.024^{a}
TU 500 mg L^{-1}	7.96 ± 0.50^{bc}	434.9 ±29.3^{abc}	6.57 ±0.32^{c}	1.22 ±0.021^{a}
TU 750 mg L^{-1}	8.64 ± 0.28^{ab}	452.5 ±24.9^{abc}	6.96 ±0.27^{ab}	1.24 ±0.016^{a}
TU 1000 mg L^{-1}	9.03 ± 0.52^{a}	464.6 ±34.6^{ab}	7.23 ±0.19^{a}	1.25 ±0.027^{a}
LSD (0.05)	0.711	50.4	0.380	0.140
Treatment	2nd Year			
	P_N (μ mol m^{-2} s^{-1})	g_s (m mol m^{-2} s^{-1})	E (m mol m^{-2} s^{-1})	WUE
Control	8.55 ± 0.35^{d}	404.4 ±21.8^{c}	5.52 ±0.15^{d}	1.55 ±0.058^{b}
TGA 200 mg L^{-1}	10.04 ± 0.28^{c}	464.7 ±18.6^{b}	5.96 ±0.07^{c}	1.69 ±0.032^{a}
TGA 300 mg L^{-1}	10.48 ± 0.44^{abc}	499.1 ±14.1^{ab}	6.11 ±0.18^{bc}	1.72 ±0.079^{a}
TGA 400 mg L^{-1}	11.48 ± 0.42^{a}	518.3 ±17.9^{a}	6.60 ±0.14^{a}	1.74 ±0.040^{a}
TU 500 mg L^{-1}	10.36 ± 0.49^{bc}	472.0 ±15.4^{b}	6.05 ±0.11^{c}	1.71 ±0.054^{a}
TU 750 mg L^{-1}	10.83 ± 0.46^{abc}	492.0 ±16.3^{ab}	6.27 ±0.15^{abc}	1.73 ±0.042^{a}
TU 1000 mg L^{-1}	11.21 ± 0.44^{a}	513.3 ±20.0^{a}	6.45 ±0.14^{ab}	1.74 ±0.067^{a}
LSD (0.05)	0.908	39.1	0.313	0.066

Values followed by the different letters in each column are significantly different at $P \leq 0.05$ according to LSD.
TGA: Thioglycollic acid; TU: Thiourea; P_N: Net photosynthetic rate; g_s : Stomatal conductance; E: Transpiration rate; WUE; Water use efficiency.

Photosynthetic pigment contents

Photosynthetic pigments are essential for plants to harvest light; however, a decline in their concentrations can limit the photosynthetic rate of leaves and primary production (Zhang and Kirkham, 1996). Application of PBR's significantly ($P \geq 0.05$) enhanced the contents of total Chl, Car, a/b ratio and Chl/Car ratio during both the years (Table 3). Averaged across both the years, sulphydryl compounds application had 18-30%, 9-16%, 10-17% and 9-13% higher total Chl, Car, a/b ratio and Chl/Car ratio, respectively, than the untreated plants. The greatest improvement in contents (Total Chl and Car) and ratios (a/b and Chl/Car) of photosynthetic pigments were recorded for plants treated with TU 1000 mg L^{-1}. Decrease in chlorophyll content under water deficit stress is attributed to the oxidation of chlorophyll and damaged ultra-structure of chloroplast (Anjum *et al*., 2011). The present study revealed the exogenous application of PBR's via foliar application helped to maintain the total Chl, carotenoid contents and hence, mitigated the adverse effects of drought stress. 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 (Nathawat *et al*., 2007b), in cluster bean (Garg *et al*., 2006) and in moth bean (Nathawat *et al*., 2018). This appears logical because sulphydryl compounds exhibit cytokinin- like activity, and is known to delay leaf senescence (Vassilev and Mashev, 1974). The final stage of leaf development is inevitable senescence with a decline in physiological activity. The stability of photosynthetic components could be attributed by maintenance of positive leaf LRWC under stress as a result of osmotic adjustment (Basu *et al*., 2007; Burman *et al*., 2008).

Antioxidant enzyme activity

The induction of antioxidant enzymes activities is a general defence mechanism that

Table 3. Effects of foliar application of sulphydryl compounds on total chlorophyll (Total Chl), carotenoid (Car), a/b ratio, Chl/Car ratio and glutathione-S-transferase (GST) of cluster bean

Treatment	1st Year				
	Total Chl ($mg.g^{-1}$FW)	Car ($mg.g^{-1}$FW)	a/b ratio	Chl /Car ratio	GST (µmol min^{-1}. mg^{-1} protein)
Control	2.06 ± 0.11^{d}	1.06± 0.02^{d}	1.65 ±0.010^{c}	1.94 ± 0.04^{b}	46.7 ±0.86^{e}
TGA 200 mg L^{-1}	2.52 ± 0.09^{bc}	1.17±0.04^{bc}	1.85 ±0.04^{b}	2.16 ± 0.03^{a}	58.9 ±1.55^{c}
TGA 300 mg L^{-1}	2.64 ± 0.18^{ab}	1.21±0.01^{abc}	1.90 ±0.03^{ab}	2.18 ± 0.04^{a}	62.4 ±1.51^{b}
TGA 400 mg L^{-1}	2.72 ± 0.12^{a}	1.25±0.05^{a}	1.95 ±0.07^{a}	2.18 ±0.02^{a}	65.9 ±1.30^{a}
TU 500 mg L^{-1}	2.42 ± 0.13^{c}	1.15±0.06^{c}	1.83 ±0.05^{b}	2.12 ±0.05^{a}	56.2 ±1.78^{d}
TU 750 mg L^{-1}	2.68 ± 0.06^{ab}	1.22±0.01^{ab}	1.92 ±0.04^{ab}	2.19 ±0.01^{a}	59.6 ±1.61^{c}
TU 1000 mg L^{-1}	2.77 ±0.05^{a}	1.26±0.02^{a}	1.96 ±0.05^{a}	2.19 ±0.03^{a}	67.7 ± 1.58^{a}
LSD (0.05)	0.178	0.058	0.090	0.080	1.6

Treatment	2nd Year				
	Total Chl ($mg.g^{-1}$FW)	Car ($mg.g^{-1}$FW)	a/b ratio	Chl/Car ratio	GST (µmol min^{-1}. mg^{-1} protein)
Control	1.93±0.08^{b}	0.73±0.01^{b}	1.39±0.03^{c}	2.64±0.04^{b}	56.7±3.0^{c}
TGA 200 mg L^{-1}	2.26±0.09^{a}	0.79±0.02^{a}	1.54±0.02^{ab}	2.86±0.06^{a}	76.9±3.5^{b}
TGA 300 mg L^{-1}	2.36±0.09^{a}	0.81±0.01^{a}	1.57±0.03^{ab}	2.91±0.05^{a}	80.4±4.0^{ab}
TGA 400 mg L^{-1}	2.39±0.06^{a}	0.82±0.01^{a}	1.58±0.04^{ab}	2.94±0.05^{a}	84.9±3.6^{a}
TU 500 mg L^{-1}	2.28 ±0.06^{a}	0.80±0.02^{a}	1.52 ±0.03^{b}	2.86±0.03^{a}	79.2±3.9^{b}
TU 750 mg L^{-1}	2.32 ±0.07^{a}	0.81±0.01^{a}	1.54 0.05^{ab}	2.87±0.06^{a}	81.8±4.8^{ab}
TU 1000 mg L^{-1}	2.42±0.06^{a}	0.82±0.02^{a}	1.61 ±0.04^{a}	2.96±0.05^{a}	87.9±4.2^{a}
LSD (0.05)	0.163	0.029	0.072	0.101	7.94

†Values followed by the different letters in each column are significantly different at $p \leq 0.05$ according to LSD.
TGA : Thioglycollic acid; TU: Thiourea; Total Chl : Total chlorophyll; Carotenoid: Car; Chlrophyll a and chlorophyll b ratio: a/b ratio.
Chlorophyll and carotenoid ratio: Chl / Car ratio; Glutathione -S- transferase: GST.

plants use against drought stress; it helps them to overcome oxidative stress and associated cell damage from it. Therefore, under drought stress, application of sulphydryl compounds improves the antioxidant defence mechanism by unregulated glutathione-S-transferase activity in clusterbean, which was efficient enough to remove reactive oxygen species (ROS) with respect to untreated plants (Table 3). The maximum GST activity was recorded with application of TU 1000 mg L^{-1} which was 50% higher than the control (Table 3**).** Enhancement of antioxidant enzymes with the application of sulphydryl compounds might be attributed to increased synthesis of enzymes due to supply of thiol (-SH) containing compounds (Loggini *et al*., 1999).

Gas-exchange parameters

Photosynthesis is important traits for assessment of drought tolerance, and widely affected by environmental stress conditions. The response of plants with reduction in transpiration rate, suggests water conservation from partial stomatal closure and reduced loss of water through stomata (Jones *et al*., 1985). Reduction in photosynthetic rate under drought stress has been related to stomatal limitation due to stomatal closure (Rodrigues *et al*., 1993). The effects of application of sulphydryl compounds on gas exchange parameters at post-flowering stage are presented in Table 3. Application of sulphydryl compounds significantly ($P \geq 0.05$) increased the net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and WUE during both the years. Averaged across both the years, PBR's application had 18-36%, 20-39% and 9-21% higher P_N, g_s and *E* respectively, compared with untreated plants. The WUE, measured the ratio between P_N and E, showed significantly ($P \geq 0.05$) response to PBR's application and treated plants exhibited 9-12% higher values of WUE than that of untreated plants. The plant treated with TU 1000 mg L^{-1} had the greatest P_N, g_s and E values followed by TGA 400 mg L^{-1}. Our results

Table 4. Effects of foliar application of sulphydryl compounds on yield components and yield of cluster bean

Treatment	1st Year				
	NP (m^{-2})	NS (m^{-2})	100 - seed weight (g)	SY (ton ha^{-1})	ABY (ton ha^{-1})
Control	1784±76^{d}	12705±141^{d}	2.94 ±0.04^{a}	1.01±0.045^{c}	3.78 ±0.218^{c}
TGA 200 mg L^{-1}	2071±99^{c}	13583±314^{c}	2.99 ±0.05^{a}	1.16 ±0.043^{b}	4.32 ±0.206^{b}
TGA 300 mg L^{-1}	2171±61^{bc}	14147±289^{ab}	3.04 ±0.03^{a}	1.21 ±0.041^{ab}	4.63 ±0.193^{ab}
TGA 400 mg L^{-1}	2218±91^{abc}	14303±196^{a}	3.06 ±0.05^{a}	1.25 ±0.043^{a}	4.78 ±0.257^{a}
TU 500 mg L^{-1}	2093± 64^{abc}	13825±356^{bc}	3.01 ±0.02^{a}	1.17 ±0.047^{b}	4.54 ±0.217^{ab}
TU 750 mg L^{-1}	2131± 58^{ab}	13944±210^{bc}	3.07 ±0.04^{a}	1.22 ±0.053^{ab}	4.63 ±0.250^{ab}
TU 1000 mg L^{-1}	2183± 79^{a}	14651±300^{a}	3.12 ±0.03^{a}	1.23 ±0.046^{ab}	4.82 ±0.226^{a}
LSD (0.05)	119	407	NS	0.07	0.32
Treatment	2nd Year				
	NP (m^{-2})	NS (m^{-2})	100 - seed weight (g)	SY (ton ha^{-1})	ABY (ton ha^{-1})
Control	1683±72^{c}	11512±356^{d}	2.91 ±0.06^{a}	0.97 ±0.035^{c}	3.65 ±0.104^{c}
TGA 200 mg L^{-1}	1921± 99^{b}	12412±320^{c}	2.97 ±0.05^{a}	1.09 ±0.043^{b}	4.11 ±0.118^{b}
TGA 300 mg L^{-1}	1996± 57^{ab}	12917±301^{abc}	2.97 ±0.04^{a}	1.14 ±0.036^{ab}	4.24 ±0.103^{ab}
TGA 400 mg L^{-1}	2043±69^{ab}	12968±350^{a}	3.07 ±0.05^{a}	1.15 ±0.033^{a}	4.32 ±0.146^{ab}
TU 500 mg L^{-1}	1943± 64^{ab}	12561±269^{bc}	2.95 ±0.04^{a}	1.11 ±0.021^{b}	4.17 ±0.82^{ab}
TU 750 mg L^{-1}	1981±58^{ab}	12792±272^{abc}	2.98 ±0.04^{a}	1.13 ±0.026^{ab}	4.27 ±0.117^{ab}
TU 1000 mg L^{-1}	2008± 70^{a}	13104±348^{a}	3.09 ±0.06^{a}	1.15 ±0.032^{a}	4.36 ±0.107^{a}
LSD (0.05)	105	492	NS	0.06	0.24

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

indicated that sulphydryl application improved photosynthetic parameters (P_N, g_s sans E) compared with untreated plants. The higher P_N for -SH treated plant might be explained by the higher content of photosynthetic pigments, less water deficit-induced oxidative damage to metabolic activity reflected by higher GST activity (Table 2). Exogenous application of PBR's often results in enhanced efficiency of photosystems in quenching better quantum yields with improved photosynthetic rates and efficient photo assimilate translocation between source and sink (Pandey *et al*., 2013; Nathawat *et al*., 2021). The enhancement in gs and E with sulphydryl compounds application could be attributed to their ability to maintain favorable water balance reflected by higher LRWC and ψ_w (Fig. 1). The improved total Chl and LRWC increased rate of photosynthesis and transpiration in clusterbean plants sprayed by exogenous application of TGA and TU (Table 2 and Fig. 1) can be correlated to the expression of osmotic adjustment, cell membrane integrity, and antioxidants of cell and by enhanced tissue water status in leaves due to foliar applicants (Farooq *et al*., 2009; D'Souza *et al*., 2009). Thus, PBRs have greater role in maintaining leaf water status and photosynthetic efficiency for crop grown under drought conditions. These results suggested that these traits greatly contributed in enhancement of grain yield with application of PBR's especially TGA 400 mg L^{-1} and TU 1000 mg L^{-1} by maintaining leaf water status by closing stomata, increasing relative water content, water potential and cell turgor pressure under water stress condition.

Yield attributes and yield

The effects of application of sulphydryl compounds on yield attributes (pods m^{-2}, seed m^{-2} and seed weight) and yield (seed yield ton ha^{-1} and biomass yield ton ha^{-1}) are presented in Table 4. Application of PBR's had significantly effects on pods m^{-2} and seed m^{-2} during both the years. The pods m^{-2} varied from 1683-2218 m^{-2} and seed ranged from 11512-14651 m^{-2}. Application of PBR's did not exert any significant effect on 100 seed weight. The application of PBR's was recorded higher pods m^{-2} than control and increase was found 23% and 21% with TGA 400 mg L^{-1} and TU 1000 mg L^{-1} foliar application, respectively.

Averaged across both the years, SY ton ha^{-1} and ABY ton ha^{-1} was significantly ($P \geq 0.05$) higher with the foliar sprays of the PBR's compared to the control. The mean SY varied from 0.99-1.20 to ha^{-1} during both the years. Application of TGA mg L^{-1} gave the highest SY, with an increase 21% compared to the control. ABY mean varied from 3.72-4.59 ton ha^{-1} during both the years. Foliar application of TU 1000 mg L^{-1} produced 24% and 22% higher ABY than control respectively. Significant improvement in yield attributes (pods m^{-2} and seed m^{-2}) was due to greater photosynthetic efficiency and better partitioning of photosynthesis (Burman *et al*., 2004; Grag *et al*., 2008; Nathawat *et al*., 2018). The enhancement of photosynthesis (Ramaswamy *et al*., 2007; D'souza *et al*., 2009) and better partitioning of photosynthates to sink for various compounds have been reported earlier under abiotic stress conditions (Sahu and Singh, 1995; Srivastava *et al*., 2008). The protective mechanism of PBR's regulates the root growth for improving plant water status, photosynthetic efficiency and source-sink homeostasis resulting enhanced yield and metabolism for overall improvement in plant growth (Srivastava *et al*., 2016).

Conclusion

Exogenous application of bio-regulators ameliorates clusterbean crop performance under water stress. This ameliorative effect was associated with the alteration of physio-biochemical process such as enhanced growth parameters, water status, photosynthetic pigments content, gas exchange parameters and yield traits. Foliar spray of -SH compounds (thiourea and thioglycolic acid) can help to increase the yield of clusterbean under stress 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., Xie, X., Wang, L., 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

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

Barrs, H.D. and Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Australian Journal of Biological Sciences* 15: 413-428.

Basu, P.S., Masood, A. and Chaturvedi, S.K. 2007. Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian journal of experimental biology 45(3): 261-267.

Boutraa, T. 2010. Improvement of water use efficiency in irrigated agriculture: A review. *Journal of Agronomy* 9: 1-8.

Bray, E.A. 1997. Plant responses to water deficit. *Trends in Plant Science* 2: 48-54.

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.

Burman, U., Garg, B.K. and Kathju, S. 2008. Influence of pre-and post-drought application of thiourea on growth, net photosynthesis and nitrogen metabolism of cluster bean. *Annals of Arid Zone* 47: 177-184.

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.

Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M. and Feller, U. (2009). Drought stress effects on rubisco in wheat: changes in the rubisco large subunit. Acta Physiologiae Plantarum. 31: 1129-1138.

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 rainfed 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.

Haloi, B. and Baldev, B. 1986. Effect of irrigation on growth attributes in chickpea when grown under different dates of sowing and population pressure. *Indian Journal of Plant Physiology* 29: 14-27.

Hiscox, J.D. and Israelstam, G.F. 1979. A method for extraction of chloroplast from leaf tissue without maceration. *Canadian Journal of Botany* 57: 1332-1333.

Hunt, R. 1978. *Plant Growth Analysis*, Edward Arnold, U.K. pp. 26-38.

Jones, M.B., Clifton-Brown, J., Raschi, A. and Miglietta, F. 1995. The effects on *Arbutus unedo* L. of long-term exposure to elevated CO_2. *Global Change Biology* 1: 295-302.

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.

Mannervik, B. and Guthenberg, C. 1981. Glutathione transferase (human placenta). *Methods in Enzymology* 77: 231-235.

Mudgil, D., Barak, S. and Kharkar, B.S. 2014. Guar gum processing properties and food applications - A review. *Journal of Food Science and Technology* 51: 409-418.

Nathawat, N.S., Kuhad, M.S., Goswami, C.L, Patel, A.L. and Kumar, R. 2007 a. Interactive effect of nitrogen sources and salinity on growth Indices and ion content of Indian mustard. *Journal of Plant Nutrition* 30(4): 569-598.

Nathawat, N.S., Rathore, V.S., Meel, B., Bhardwaj, S. and Bhargava, R. 2018. Exogenous sulphydryl improves membrane stabilization, photosynthesis and antioxidant systems in *Vigna aconitifolia* L. under water stress. *Proceeding of the national Academy of Sciences, India, Section B: Biological Sciences* 88(3): 875-885.

Nathawat, N.S., Rathore, V.S., Meel, B., Bhardwaj, S. and Yadava, N.D. 2016. Enhancing yield of clusterbean (*Cyamopsis tetragonoloba* L. Taub) with foliar application of sulphydryl compounds under hot arid conditions. *Experimental Agriculture* 52(3): 418-433.

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

Nathawat, N.S., Rathore, V.S., Soni, M.L., Singh, J.P. and Yadava, N.D. 2021. Exogenous application of sulphydryl compounds enhances growth, photosynthetic efficency and yield of mothbean (*Vigna aconitifolia* L.) under water limiting environment. *Legume Research* 44(1): 67-73.

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.

Nomami, H. 1998. Plant water relations and control of cell elongation at low water potentials. *Journal of Plant Research* 111: 373-382.

Pandey, M., Srivastava, A.K., D'Souza, S.F. and Penna, S. 2013. Thiourea, a ROS scavenger, regulates source-to-sink relationship to enhance crop yield and oil content in *Brassica juncea* (L.). *PLOS One* 8: Article Number: e73921.

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

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.

Rodrigues, M.L., Chaves, M.M., Wendler, R., David, M.M., Quick, W.P., Leegood, R.C., Stitt, M. and Pereira, J.S. 1993. Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. *Australian Journal of Plant Physiology* 20: 309-321.

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

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

Shao, H.B., Chu, L.Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R. and Shao, M.A. 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the eco environment in arid regions of the globe. *Critical Reviews in Biotechnology* 29: 131-151

Sharma, K.M., Sharma, D.D., Shukla, K.B. and Upadhyay, B. 2008. Growth partitioning and productivity of wheat as influenced by fertilization and foliar application of bioregulators. *Indian Journal of Plant Physiology* 13(4): 387-393.

Solanki, N.S. and Sahu, M.P. 1991. Productivity and P-use efficiency of clusterbean (*Cyamopsis tetragonoloba*) as influenced by bio-regulators and phosphorus. *Indian Journal of Agronomy* 52(2): 143-147.

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.

Srivastava, A.K., Ratnakumar, P., Minhas, P.S. and Suprasanna, P. 2016. Plant bioregulators for sustainable agriculture; integrating redox signaling as a possible unifying mechanism. *Advances in Agronomy* 137: 237-278.

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.

Williams, R.F. 1948. The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants. *Australian Journal of Science and Research* 1B: 333-361.

Zhang, J. and Krikham, M.B. 1996. Antioxidant response to drought in sunflower and sorghum seedlings. *New Phytologist* 132: 361-373.

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